

FATE OF NITROGEN AND METALS FOLLOWING ORGANIC WASTE
APPLICATIONS TO SOME FLORIDA SOILS

By

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Increased production of organic wastes in municipalities throughout the United States, in addition to the ban of and lack of popularity for some disposal methods, has created a need to increase our understanding of the consequences of waste applications to croplands.

The work included in this dissertation has attempted to answer several questions: Does the application of 7 to 8 Mg ha⁻¹ sewage sludge biosolids to a mature citrus grove result in significant accumulations of heavy metals in soil and plant tissues? Do additions of organic amendments increase the potential for denitrification in sandy soils? If so, how long does the effect persist, and how do soil moisture content, temperature and electron availability affect the rate of denitrification in waste-amended and unamended sandy soils?

Studies were conducted in Palm Beach, Manatee, and Okeechobee counties, Florida, with the elemental concentrations of the organic amendments used during each study testing below the maximum allowable concentration for unrestricted use. Heavy metals and nutrients in the soil (including depth effects), citrus leaves, juice and peel were monitored for plots amended with sewage sludge biosolids during four years in Palm Beach County. In part due to considerable variability in age and health condition for the grove, results showed no significant increase in levels of the majority of plant nutrients and metals for either soil or plant parts.

Applications of organic amendments tended to increase the maximum potential rate of denitrification at each location in comparison to control plots, with results persisting for as much as 12 months thereafter. Highest potential denitrification rates were observed for a plastic-mulched bed planted to tomato (*Lycopersicon esculentum* Mill.), largely due to the proximity of the water table (0.45 m). Rates measured for intact soil cores from beds amended with an equivalent rate of 30 Mg ha⁻¹ municipal solid waste compost oscillated between 1.6 and 7 g N ha⁻¹ hr⁻¹ while, for control plots, they oscillated between 1.4 and 1.9 g N ha⁻¹ hr⁻¹. Intact soil-core rates measured at a citrus site near the town of Okeechobee were nearly 10 times lower than those measured at a tomato site near Bradenton. Samples collected at the Okeechobee site, with a water table depth of 1- 1.5 m, had relatively low soil moisture content. This may explain the low rates of gas production in this case.

Soil aeration appears to be the main regulator of denitrification in the soils under study. Temperature also appeared to affect the rate of denitrification, but new methods to

study this effect should be developed. Rates of denitrification also tended to increase with increasing concentrations of soluble organic carbon and nitrate.

CHAPTER 1 INTRODUCTION

More than five million metric tons of sewage sludge and 270 million metric tons of municipal solid waste (MSW) are generated in the United States each year. The amount of waste will increase in the future due to normal population growth and to the fact that increasing numbers of households will be connected to publicly-owned wastewater treatment plants.

Traditional methods for waste disposal, such as incineration, ocean dumping, and landfilling, are becoming increasingly more costly, and some of them are now banned. Although there is no method for disposal of organic wastes that guarantees 100% safety, land application is a viable alternative which has been practiced for centuries. In the late 1800s, sewage farms were established in England to receive the discharges of wastes from the city. In China, the use of "night soil" as a soil amendment is well-known. In the United States, sewage sludge has been land-applied in California, Ohio and Maryland since the turn of the century.

Municipal solid waste has also been applied to land since the beginning of this century. In the early 1900s, Sir Albert Howard developed a composting procedure known as the "Indore System." In the 1940s and the 1950s, studies were conducted to elucidate

the scientific principles of composting. Furthermore, with the passage of the 1965 Federal Solid Wastes Act, research on composting received considerable financial and public support.

Application of MSW compost to agricultural land has traditionally been better-accepted than the application of sewage sludge. Organic wastes, in addition to being a source of plant nutrients, also contain a number of organic and inorganic compounds and pathogens which, if found in sufficient numbers, constitute a hazard for human, livestock, and crop health.

In 1993, the U.S. Environmental Protection Agency (EPA) promulgated the Part 503 Sludge Rule. Although the rule was developed originally for sewage sludge use, some states have adopted the same parameters for MSW compost use as well. The objective of this rule is to reduce the concentration of pollutants entering wastewater treatment plants, in addition to providing acceptable handling practices and delineating annual and cumulative loadings of heavy metals to sludge-amended soils. The Part 503 Rule is based on a risk-assessment approach that considers the potential detrimental effects of pollutants on humans, animals, crops and the environment. The regulations are based in turn on more than 20 years of research and experience with applying organic wastes to agricultural land, especially with respect to agronomic crops and some non-commercial species in the Western and Midwestern regions of the United States.

If all of the sewage sludge produced in the state of Florida were to be land-applied, 12 percent of the state's agricultural land would be required. In Florida, organic amendments are being used in containerized operations as well as in vegetable production,

where they are being applied between production beds. Several trials have been conducted to demonstrate the benefits of irrigating citrus groves with wastewater. In fact, the practice of irrigating crops with reclaimed water in Florida is well-accepted. Few studies have been conducted, however, to evaluate the potential use of sewage sludge in mature citrus groves.

Land application of organic amendments can change the physical and chemical properties of a soil. Additions of organic carbon promote microbial growth and consequently will affect, directly or indirectly, microbially-mediated processes such as the mineralization of nutrients or the loss of nitrogen through denitrification.

Denitrification is a form of microbial respiration under oxygen-depleted conditions where bacteria use nitrate instead of oxygen as the terminal electron acceptor. Its end products are nitrous oxide and nitrogen gas. Denitrification is an important pathway for nitrogen transformations, with both economic and environmental implications. Researchers have long studied denitrification, aiming to reduce losses of nitrogen from agricultural land. There is also concern that gaseous nitrogen forms are contributing to atmospheric ozone depletion. In wastewater treatment plants and in soils, denitrification could remove excess nitrate that otherwise might end up in drinking waters. Nitrate concentrations in drinking waters above the maximum recommended by the U.S. Health Department, and reiterated by the USEPA, set at 10 mg L^{-1} nitrate-N, have been related to toxicity in infants. Livestock standards have also been established, though at higher levels.

Denitrification in agricultural soils is regulated by the oxygen, nitrate, and soluble organic carbon concentrations in each soil. Temperature, soil pH, and salinity also can influence the rate of denitrification.

In Florida, a large portion of the state's cropland occurs as sandy soils of inherently low fertility and yet differing natures. Soils of the central ridge are deep sands (Entisols) with the water table generally found several meters deep. The soils are prone to the leaching of excess fertilizer, primarily in nitrate form. Denitrification is not generally a major factor, since most of the profile remains aerobic throughout the year. Additionally, the concentrations of soluble organic carbon or of appropriate microbes near the water table are not generally adequate for denitrification to proceed. However, additions of organic amendments to the top soil layers may increase the potential for denitrification, especially after irrigation or rainfall events.

An important segment of the state's cropland is also found in soils with a high water table, typically only 40 to 60 cm deep. Many of the vegetables grown along the west coast of Florida are irrigated by maintaining a perched water table at the 45 cm to 60 cm depth. Additions of organic amendments, plus proximity of the water table, make these soils ideal biological reactors for the denitrification process. Estimates of denitrification rates under the above conditions remain scarce or nonexistent, however.

The objectives of this dissertation are:

- 1) To generate data showing that sewage sludge at a rate of 7 to 8 Mg ha⁻¹ year⁻¹ can be safely applied to a mature citrus grove, without significant accumulation of heavy metals in soil and plant parts;

- 2) To study the effect of organic amendments on the rate of denitrification in sandy soils under different cultural practices; and
- 3) To study the relationship between denitrification and related soil parameters in a sandy soil.

CHAPTER 2 LITERATURE REVIEW

Introduction

Land application of organic waste has been a common cultural practice by farmers around the world for centuries. However, as recently as the early 1970s, a considerable amount of research was initiated to study crop and soil responses to organic waste applications. This increase in research was largely a result of the 1977 amendment to the 1972 Federal Water Pollution Control Act, which resulted in the Clean Water Act of 1977.

In the 1980s and 1990s, the U.S. Environmental Protection Agency (USEPA) developed a series of regulations and guidelines for the safe use of sewage sludge biosolids and septage. These regulations included publication of the Part 503 Sludge Rule, which set limits for the cumulative loading of heavy metals to soil.

"Organic waste" is a general term used to describe a wide variety of discarded products. Such products include domestic and municipal wastes that come from residential, commercial, industrial, and agricultural areas, and also agriculture-related wastes including discarded products resulting from raising and processing animal and crop-related products.

Sewage Sludge

Sewage sludge, "sludge," or "biosolids" is the solid byproduct of municipal wastewater processing. Sewage sludge consists mainly of particulate organic matter and associated plant nutrients, heavy metals, and other organic and/or toxic compounds. Sludge can be produced either as slurry or as dewatered solids.

Municipal Wastewater and Sludge Treatment

Conventional wastewater treatment includes preliminary, primary, and secondary treatment (Fig. 2.1). Most sewage treatment plants produce a secondary-treatment product at present. Tertiary treatment is needed when there is a requirement for higher quality effluent than produced by conventional secondary wastewater treatment.

Preliminary wastewater treatment. Once municipal wastewater enters a sewage treatment plant, it is screened for constituents that may cause maintenance or operational problems. Such constituents include rags that could interfere with the equipment; and heavy, inorganic, sand-like solid materials that could interfere with subsequent processes (National Research Council, 1996).

Primary wastewater treatment. Preliminary effluent is held in a clarification tank, where more than one-half of the suspended solids and about one-third of the biochemical oxygen demand (BOD) are removed by a combination of sedimentation and decomposition. Primary wastewater treatment concurrently reduces the concentrations of nutrients, heavy metals, pathogens, and other toxic compounds that can be absorbed by, or entrained within, the associated solids. A typical treatment plant produces 2500 to

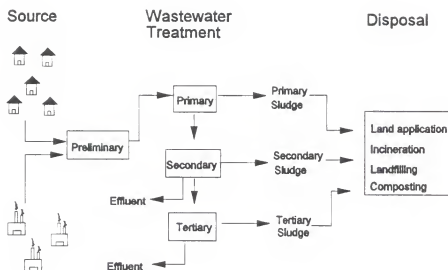


Fig. 2.1. Flow chart for a typical wastewater treatment plant. (Adapted from USEPA, 1984).

3000 L of "primary sludge" for each million L of wastewater (Hue, 1995).

Secondary wastewater treatment. Suspended solids and biodegradable compounds not removed during primary settling are removed during secondary treatment by means of biological processes. The most common biological processes employed at this stage include "activated sludge" and "trickling filters." Activated sludge refers to a microbial population kept in suspension that oxidizes a considerable fraction of the soluble and colloidal organic matter (Vesilind and Peirce, 1985). This process causes microbial colonies to grow, flocculate and form additional settleable particles. Trickling filters usually consist of solid media that support microbial films. These films extract organics

and nutrients from wastewater that trickles over it. The resultant residue is called "waste-activated sludge".

Tertiary wastewater treatment. The most common type of tertiary treatment consists of disinfection for control of pathogens and viruses, along with the removal of persistent organic compounds and some of the plant nutrients. Activated carbon is commonly used to remove organics, while nitrification-denitrification is used to remove nitrogen, and microbial uptake and chemical precipitation are used to remove phosphorus (National Research Council, 1996).

Sludge treatment processes. There are also several options to handle the sludge produced during the various wastewater treatment processes (Fig. 2.2). The first option is directed at reducing volume and includes "thickening," "dewatering," "conditioning," and "drying" (Tchobanoglous and Burton, 1991). A second treatment process includes biological and chemical stabilization. Biological stabilization includes composting and anaerobic digestion, while chemical stabilization refers mainly to pH control.

Sludge Production in the United States

Population growth and the associated increase in urbanization, together with enactment of the Clean Water Act, have resulted in an increase in the volume of sewage sludge generated in the United States over the past two decades. It is estimated that 75% of the population is now served by publicly-owned treatment works (POTWs), with the other 25% still being served by household septic systems (USEPA, 1995).

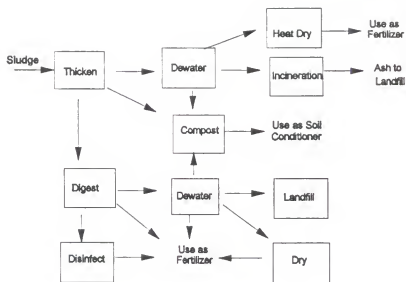


Fig. 2.2. Sludge handling alternatives (National Research Council, 1996).

In 1984, the United States population generated approximately 6 million dry Mg of sewage sludge; by 1990 the production of sludge had increased by 37%. It is estimated that, by the year 2000, the generation of sludge will have doubled to 12 million dry Mg of sewage sludge (Hasbach, 1991). Henry and Heinke (1989) estimated that a city of 200,000 people produces nearly 120,000 cubic meters of wastewater per day. European nations, with a population of 345 million in 1993, generated approximately 6.5 million dry Mg of sewage sludge, much of it in Germany (Fig. 2.3). It is projected that, by the year 2000, the production of sewage sludge in the European Union will be approximately 9 million dry Mg (Hall, 1995).

Sewage Sludge Characteristics

Nutrient content. Sewage sludge contains significant amounts of nutrients essential for plant growth, with nitrogen (N) and phosphorus (P) present in the greatest quantities. In fact, the concentration of N and the rate of organic-N mineralization are two of the most important factors that determine the rate of sewage sludge that can be safely land-applied. The annual rate of mineralization of sludge-borne organic nitrogen can vary between 10 and 50% (Parker and Sommers, 1983), and depends on factors such as the

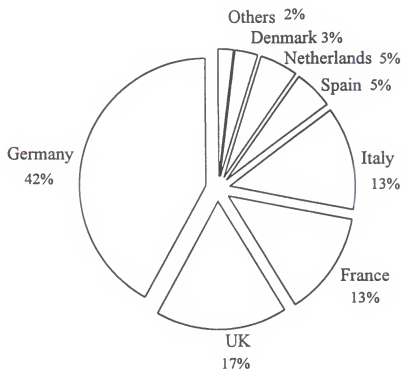


Fig. 2.3. Sludge production in the European Union (Hall, 1995).

initial organic-N content, type of sludge, and soil and climatic conditions (Sommers et al., 1976).

The concentration of total N and other plant nutrients in sewage sludge varies widely (Table 2.1). Nitrogen, for instance, has been found to vary from 0.1 to 17.6 %, with a mean of 3.9% and a median of 3.3%. Phosphorus, unlike N, is quite insoluble and therefore concentrates in the organic and inorganic solid phases to form iron aluminum phosphates and/or iron and aluminum hydrous oxides (Sommers, 1977). Potassium content of sewage sludges is normally low, typically less than 1%, due to this element's high degree of water solubility (Dowdy et al., 1976). Potassium tends to remain in the wastewater effluent or in the soluble fraction of the sludges. Sewage sludges are also a source for other plant nutrients including calcium, iron, and magnesium. When sludges are applied as a sole source of N, these nutrients are generally present in sufficient amounts to meet crop nutrient requirements.

Table 2.1. Concentration ranges and typical concentrations of nutrients in sewage sludges from the U.S. (National Research Council, 1996).

Nutrient	Sewage Sludge Range (%)	Typical Concentration (%)
Nitrogen	0.1-17.6	3.0
Phosphorus	0.1-14.3	1.5
Potassium	0.02-2.6	0.3
Calcium	0.1-25	4
Magnesium	0.03-2.0	0.4
Sulfur	0.6-1.5	1.0
Iron	0.1-15.3	1.7

Heavy metals. Heavy metals concentrations in sewage sludges can be highly variable due to seasonal effects and geographical location of the wastewater plant. Plants receiving primarily industrial wastes produce sludges with higher metal concentrations than plants receiving mainly residential wastes. The concentration of metals can also be influenced by the volume of flow at the treatment plant over time. The variability in heavy metals concentrations has considerably decreased over the last 20 years, due mainly to an improvement in U.S. pollution control programs (Hue, 1995). Table 2.2 shows a summary of two surveys: one conducted in 1977 (Sommers, 1977) with data for total metals concentrations in sludges coming primarily from the north-central United States, and a second one conducted in 1990 by the U.S. Environmental Protection Agency that analyzed sludges from 239 plants located throughout the United States (Kuchenrither and Carr, 1991). The ranges in metals concentrations varied by several orders of magnitude, with concentrations that fell near the high end of the concentration range consistently representing industrial sources. The ranges in metal concentrations during the 1990 study were not as wide as the ranges during 1977 for most of the metals. Concentration means and medians calculated from the 1990 data were generally smaller in magnitude than values calculated from the 1977 data. The difference in metals concentrations is probably a result of industrial pretreatment programs that are now enforced. There is also a clear difference between the mean and median values for the concentration of each of the metals in both studies. This gives an indication of population skewness and, for that reason, the median is probably a more appropriate parameter from which generalizations regarding the metals concentrations of sewage sludges can be drawn.

Table 2.2. Concentrations of selected metals in sewage sludge samples collected from 150 treatment plants in the north-central U.S. (1977 study) or from 239 treatment plants located throughout the U.S. (1990 study).

Metal	Range Study I [†]	Range Study II [‡]	Mean Study I	Mean Study II	Median Study I	Median Study II
		----- mg kg ⁻¹ -----				
Pb	13 - 19,700	94 - 1,670	1,360	195	500	106
Zn	101 - 27,800	37.8 - 68,000	2,790	1,693	1,740	725
Cu	84 - 10,400	6.8 - 3,120	1,210	665	850	463
Ni	2 - 3,520	2 - 976	320	77	82	29
Cd	3 - 3,410	0.7 - 8,220	110	65.5	16	7
Cr	10 - 99,000	2 - 3,750	2,620	258	890	40

[†] Study I refers to Sommers (1977).

[‡] Study II refers to USEPA (1990).

Metals in sewage sludges are present in organic as well as in inorganic forms, with metals present in organic form being generally adsorbed to complexing sites on organic matter. Inorganic forms, on the other hand, may be present as phosphates, carbonates or silicates; as solid solutions with Fe, Al, or Ca; or strongly adsorbed to Fe, Al, or Ca minerals (Corey et al., 1987).

Other sludge compounds. In addition to plant nutrients and metals, sludges also contain toxic organic compounds such as polychlorinated biphenyls (PCBs) and poly- or mono-cyclic aromatic hydrocarbons (PAHs), though they normally occur below detectable limits (O'Connor et al., 1991). Bacteria, viruses and protozoa are also present in sludges, with the number and type of microorganism depending basically on the

treatment process and on the source of wastewater (Hue, 1995).

Disposal Methods

The handling and disposal of sludges is carried out under strict federal and state regulations. Selection of a disposal method should include considerations designed to achieve the most efficient use of money, materials, energy, personnel and, at the same time, should be as environmentally friendly as possible. Common methods for disposal include land application, landfilling, incineration, composting, and ocean dumping.

Landfilling. An average of 16% of the sludge produced in the U.S. at present is being landfilled. States such as Missouri, Washington, and Ohio landfill only 5% of their sludge, while Florida, Nebraska, and Tennessee landfill more than 15%. In the states of New Jersey and Rhode Island, landfilling of sludge is not allowed (Goldstein, 1991). In Europe, on the other hand, approximately 40% of the sludges are landfilled (Fig. 2.4). Many countries in Europe are introducing legislation to reduce the amount of organic matter entering landfills, however. In the future for countries such as Germany, Denmark, and France, sludge will only be acceptable in landfills as incinerator ash (Hall, 1995). When landfilling sludge there are two parameters that must be monitored constantly. One is the leachate, which results from excess moisture and rainfall, and contains toxic compounds. The second parameter involves the quantification of gases generated during anaerobic decomposition of the organic materials. These gases may contribute to the greenhouse effect (Hue, 1995).

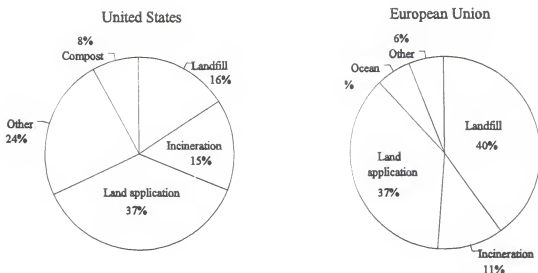


Fig. 2.4. Sewage sludge disposal methods in the U.S. and the European Union (Adapted from Hall, 1995)

Incineration. Incineration of sludge is a useful disposal method, especially in communities where land is scarce, since it reduces the sludge to less than 20% of its original volume. The states of Connecticut and Rhode Island, for instance, incinerated nearly 60% of their sludges in 1990, while Florida, Arkansas and Tennessee incinerated less than 10% during the same period (Goldstein, 1991). Incineration takes place in burners that reach temperatures between 700 and 900 °C (USEPA, 1985). Burning the sludge kills pathogens and degrades many organics, but metals are highly concentrated in the ash which must be disposed of under stringent guidelines. In Europe, all countries forecast a substantial increase in the use of incinerators. This is especially true for countries like the Netherlands and Germany, where sludge use in agriculture is becoming increasingly more difficult.

Composting. Sludge composting refers to the aerobic decomposition of organic compounds to a stable, humus-like material (Cheremisinoff, 1994). Composting is not an important disposal practice in the European Union, with less than 3% of the sludge being composted. In the U.S., approximately 8% of the sludge is being composted at present and, recently, the co-composting process of sludge and yard waste has gained popularity (Goldstein and Riggle, 1990). The biggest disadvantage of composting sludges is probably the cost associated with this process.

Ocean dumping. States like New York and New Jersey used to dispose of approximately 50% of the sludges produced by their communities via ocean dumping. However, after passing of the Ocean Dumping Ban, such practice is no longer permitted. In Europe, countries like Ireland still dumps up to 35% of their sludge in the ocean. Ocean dumping of sludge in Europe dropped by 6% over the period 1985 to 1995 (Hall, 1995), and will no longer be an alternative for disposal after 1998 when the practice will be banned by The European Community.

Land application of sludge. Land application not only represents an alternative for sludge disposal, but can also be considered a good cultural practice when growing crops or reclaiming disturbed land. Sludge is a source of heavy metals (National Research Council, 1996), and also of a majority of the nutrients required for plant growth. In addition, it contributes to improvement of some physical properties of the soil (Chaney, 1990). There are numerous studies that have shown the benefits of sludge application (Hemphill et al., 1982; Mondy et al., 1985; Sopper, 1992). Table 2.3 shows figures for the

U.S. population, sludge production, and cropland needed to dispose of the sludge from selected states as of 1992.

If sludge were to be applied at rates equivalent to $100 \text{ kg N ha}^{-1} \text{ year}^{-1}$, assuming 4% available N, states like Iowa and Kansas would require less than 1% of their agricultural area for such purposes, while Florida would need nearly 12% of its agricultural land. Land application does not represent a solution for states like Rhode Island, since close to 100% of this state's cropland would be required to dispose of its sludge (National Research Council, 1996). Based on the estimates presented in Table 2.3, it appears that land application is a generally feasible option for sludge disposal. Federal regulations for land application of sludge were first proposed in 1974, but were not made official until 1993, upon publication of the Part 503 rule (Logan, 1995). Such regulations included setting concentration limits for ten heavy metals in sludge, cumulative loading rates for those metals in soil, concentrations of the metals that can be considered to constitute "clean" sludge (Chaney, 1989), and annual loading rates that must be met if the sludge is not considered "clean" but still remains below the maximum allowable metals concentrations.

Municipal Solid Waste Compost (MSW)

Composting is a biological process that results in the partial degradation of organic wastes, and specially of the putrescible ones in household garbage and food processing wastes. Composting is also used to stabilize sewage sludge and other potential hazardous wastes.

Table 2.3. Sludge production in selected states, and the amount of cropland required to accommodate in-state sludge applications at agronomic rates (National Research Council, 1996).

Region and State	Population [†]	Sludge Produced [‡]	State Cropland
	(x 10 ⁶)	(x 1000 MT)	(%)
Pacific			
California	31.21	623	7.1
Washington	5.25	104.91	1.6
Midwest			
Iowa	5.23	104.5	0.51
Kansas	2.53	50.58	0.18
Mountain			
Colorado	3.56	71.14	0.81
Arizona	3.93	78.54	7.4
South			
Florida	13.68	273.38	11.8
Georgia	6.92	138.29	3.6
Mid Atlantic			
New York	18.70	363.63	9.7
Pennsylvania	12.05	240.80	5.6

[†]1993 estimates from the U.S. Census Bureau.

[‡]Assuming 75% of the population is connected to a sewer system.

The Composting Process

There is a wide variety of materials that can be composted under the more than ten different composting processes currently available. These processes include the use of nonstatic solids beds; static solids beds; vertical flow reactors; and horizontal and inclined

flow reactors (Stratton et al., 1995). Despite the variety of processes, all are based on principles of heat and mass transfer accompanied by adequate microbial growth (Keener et al., 1993). Considerations to be involved in the design of a composting facility include capacity and equipment required, environmental safeguards, and the amount of money available (Deener et al., 1993). Figure 2.5 shows typical flow charts for composting MSW under three different schemes. Scheme "a" results in a high-quality compost but requires additional steps that increase the operational costs. Scheme "b" is more cost-effective, while scheme "c" is the simplest compost scheme though the quality of the end product is unpredictable (Stratton et al., 1995). Under scheme "a" the material is subjected to pre-sorting to remove non-easily degradable materials such as wood, plastic, glass, and aluminum. This step results in a considerable increase in costs, since it is normally done by hand. However, in some facilities, mechanical pre-sorting may be employed. The next steps under scheme "a" include "size reduction" and "mixing". Size reduction is needed to increase surface area and promote more effective microbial activity that, in turn, will promote a faster rate of decomposition. Mixing should result in a homogeneous mixture with adequate porosity. The material can then be amended with nitrogen fertilizer if it remains high in carbon, such as paper and other cellulosic-type material. Sludge can also be added at this stage in place of inorganic N fertilizer. In some facilities, P is also added to further speed the rate of decomposition. Bulking agents such as wood chips and straw are often used to increase porosity and maintain aerobic conditions in the compost medium (Haug, 1980). Microbes can also be added, to speed the rate of decomposition and to replenish microbes killed due to the high temperatures reached during the

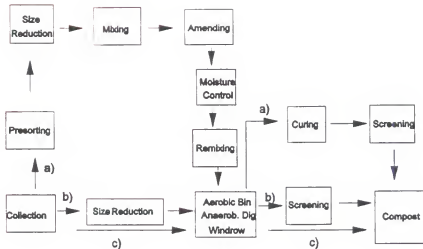


Fig. 2.5. Typical flow chart for composting MSW under three differing schemes (Adapted from National Research Council, 1996).

composting process. Moisture control is a critical part of the composting process as well. The moisture content of the compost should be between 55 and 65% of the total weight. If moisture falls below 12%, microbial activity will cease and, if it is above 70%, it can result in sticking and clogging of the facility's machinery (Golueke, 1977). Maturation and/or curing of the compost are important to prevent toxicity problems, including those reported when the compost is land-applied while still immature (Hadar et al., 1985).

Municipal Solid Waste Compost Production in The United States

In 1994, the United States generated nearly 323 million tons of solid waste (in comparison to 281 million tons in 1991), with nearly 35 million tons of the waste consisting of yard trimmings. The number of facilities composting exclusively yard

trimmings in 1994 was 3200, in comparison to 650 in 1989. Approximately 4.3 million tons were composted in 1994, which represented nearly 1.2% of the waste total (Steuteville, 1995).

MSW composting is still a fairly new practice, with only 22 operating facilities as of 1992. This lack of experience and knowledge was critical in failure of the largest compost facility in the U.S., located in Dade County, Florida. Some months later it also caused the closure of a similar facility in Oregon (Kashmanian and Spencer, 1993).

Compost Characteristics

There are a number of parameters that can affect the elemental composition, as well as the quality, of MSW compost. These parameters include source and nature of the raw material, seasonal variations, pretreatment, composting temperature, moisture content, degree of aeration, and composting duration (He et al., 1995).

Nutrients. Typical N and P concentrations in MSW compost produced in the U.S., at 1.2 and 0.3 % respectively, are lower than those found in sewage sludge but higher than for most typical surface soils of Florida. Concentrations of nutrients in composts produced in Europe are similar to those in the U.S. (Table 2.4). The high organic matter content of the compost makes it a very good conditioner with which to improve soil physical and chemical properties. Depending on soil and weather conditions, from 15 to 30% of the organic nitrogen in the compost becomes available the first year, with P, K, and Mg being even more readily available (Bidlemaier, 1993).

Heavy metals. The heavy metals concentrations in MSW compost show a high degree of temporal and spatial variability. MSW compost generally contains higher

Table 2.4. Typical total nutrient concentrations of selected MSW-composts.

	Nutrient						
	OC	N	P	K	Ca	Mg	S
	% dry-wt basis						
MSW Compost [†] (USA)	21.3	1	0.32	0.41	3.1	0.3	0.017
MSW Compost [‡] (Europe)	19.6	1.1	0.9	0.6	4.9	0.7	0.2

[†] He et al. (1995).

[‡] Bidlingmaier (1993).

concentrations of heavy metals than soils, though lower concentrations than those found in sewage sludge (Table 2.5). An exception is lead, which appears to be the most limiting heavy metal in MSW compost (Chaney and Ryan, 1993). Heavy metals are present in several forms in compost, including water-soluble, exchangeable, precipitated as discrete phases, coprecipitated in metal oxides, and adsorbed or complexed by organic compounds. These forms also may differ in terms of mobility and bioavailability, which in turn determine the potential for environmental pollution (He et al., 1995).

Other compounds in MSW-compost. Several low-molecular-weight organic acids are also commonly present in compost, especially in immature materials. Phthalates, PCBs, and PAHs have each been reported, in addition to some pesticides associated with plant residues (Chaney and Ryan, 1993). Most of these compounds are biodegradable, and may be lost during the composting process. Plant uptake of organic compounds is of significance only in the case of a few crops, such as carrots, which can accumulate considerable amounts of PAHs in the peel (Wild and Jones, 1992).

Table 2.5. Typical concentrations of selected metals in MSW-compost and sewage sludge generated in the U.S.

Element	MSW Compost [†]	Sewage Sludge [‡]	U.S. Soils [§]
	----- mg kg ⁻¹ -----		
Pb	169	106	10.6
Zn	418	725	43
Cu	107	463	18
Ni	23	29	17
Cd	2	7	0.18
Cr	33	40	---

[†] Epstein et al. (1976).

[‡] USEPA (1990).

[§] Holmgren et al. (1993).

Disposal Methods

Landfilling is the predominant means for disposing of MSW in the U.S. (Table 2.6). In 1989, more than 80% of the U.S.'s MSW was being landfilled; by 1990 that percentage had dropped to 67%; and by 1995 it had further declined to 63%. The number of landfill facilities had decreased from approximately 8000 in 1988 to 3500 in 1994 (Goldstein, 1995). The reason for this decline included new and more strict regulations, increased tipping costs, and the fact that many of those landfills had reached 100% of their capacity. In Florida, the percentage MSW being landfilled (40%) was considerably below the national average (63%) by 1995. Incineration is also a method for disposal, but its use has decreased due to classification of some of the ash as hazardous waste.

MSW compost has traditionally been used as a soil amendment in commercial vegetable production, containerized operations, pasture and turfgrass production. There

Table 2.6. Municipal solid waste generation and disposal methods for selected states in the United States during 1995 (Goldstein, 1995).

Region and State	MSW (MT yr ⁻¹)	Recycled (%)	Incinerated (%)	Landfilled (%)
Pacific				
California	45,000,000	25	2	73
Washington	7,078,000	38	6	56
Midwest				
Iowa	3,163,000	28	1	71
Kansas	3,500,000	8	0	92
Mountain				
Colorado	3,000,000	18	0	82
Arizona	4,500,000	10	0	90
South				
Florida	24,312,000	40	22	38
Georgia	8,500,000	12	3	85
Mid Atlantic				
New York	25,500,000	32	17	51
Pennsylvania	9,000,000	17	17	66
Total	322,879,000	27	10	63

are also numerous studies that have shown the benefits of MSW compost applications in crop production and forestry. For instance, MSW compost is being applied to greenhouse plants in Maryland. In Massachusetts, Minnesota, and New York, MSW is being applied to fruits, flowers and sod. In Florida, trials have been conducted with tomato, squash,

turfgrass, bell pepper, watermelon, slash pine, and citrus.

Effect of Organic Amendments on Soil Properties

The application of organic wastes to soils may result in effects that are either beneficial for plant growth or detrimental, depending on such factors as quality of the amendment, application rate, and specific soil properties including pH, organic matter content, cation exchange capacity, and texture.

Effects of Organic Amendments on Soil Physical Properties

The soil conditioning properties of organic amendments can increase the capacity of the soil to retain water through the direct effect of the organic particles in the amendments, or indirectly through effects on other physical properties including bulk density and porosity (Metzger and Yaron, 1987). Bulk density has been shown to decrease linearly with additions of organic amendments (Kladivko and Nelson, 1979; Webber, 1978). Khaleel et al. (1981) proposed a linear regression equation to show the relationship between the percent change in bulk density (ΔD_b) and the percent change in total organic carbon content (ΔC) relative to a control:

$$\Delta D_b = 3.99 + 6.62 (\Delta C) \quad R^2 = 0.69$$

The incorporation of organic amendments also contributes to improved soil aggregation and stability (Epstein, 1975). However, Chang et al. (1983) stated that, in order to affect soil physical properties, application rates higher than those commonly used may be necessary. Martens and Frankenberger (1992) observed that applications of 25 Mg ha⁻¹ increased aggregate stability by 24% with sludge, 22% with manure, 40% with alfalfa,

and 59% with straw. Hydraulic conductivity, infiltration and soil thermal properties are also affected by applications of organic amendments. There are contradictory reports on the effect of organic amendments on hydraulic conductivity under both saturated (K_{sat}) and unsaturated (K_{unsat}) conditions. Gupta et al. (1977) observed an increase in K_{sat} in a study where sewage sludge was applied to a sandy soil. Tiarks et al. (1974) reported, on the other hand, that K_{sat} in a silty clay loam soil decreased by 25% after manure applications. Infiltration was also observed to be affected in a study where a silty clay loam soil was amended (Mazurak et al., 1975), though the same workers observed no change when organic amendments were added to more coarse-textured soils.

Effect of Organic Amendments on Soil Chemical Properties

When organic amendments are added to soils, the organic portion undergoes decomposition with the products consisting of CO_2 and water. Not all the organic matter is decomposed, however, with a portion becoming part of the soil humus. This newly added organic amendment tends to increase soil cation exchange capacity (CEC). The effect of the organic amendment on CEC will tend to decrease with time as some of the more refractory organic material decomposes (Epstein et al., 1976).

Organic amendments also affect soil pH and oxidation-reduction reactions in soil. Miller et al. (1985) observed up to two units of increase in pH at an application rate equivalent to 100 Mg manure ha^{-1} . Tester (1990) observed up to three units of increase in pH when compost was applied to a sandy soil at an equivalent rate of 240 Mg ha^{-1} . The reasons for this increase probably included the high pH of the manure (pH = 7.8) and compost (pH = 7.0), and the consumption of protons during decomposition of organic

matter. Additions of organic amendments promote microbial growth, which in turn puts pressure on the oxygen concentrations in soil (electron-acceptor pressure). The oxidation-reduction potential for amended soils in the Miller et al. (1985) study was considerably lower than those for soils used as controls.

Effect of Organic Amendments on Soil Microorganisms

The application of organic amendments to soils promotes initial microbial growth due to the addition of a fresh carbon source. Long-term applications of organic amendments appear, however, to have inhibited certain strains of rhizobia in a study conducted by McGrath et al. (1994), and in other studies by Ibekwe and co-workers (1995). Ibekwe et al. (1995) also observed a 1-2 fold increase in the number of hyphal fungi, yeasts and bacteria in an amended sandy loam soil in relation to a control. In this trial, sewage sludge was applied at a rate of 14 Mg ha⁻¹ year⁻¹ over ten years. There are also reports where no effects on microorganisms were observed after organic amendments were applied (Angle and Chaney, 1989; Kinkle et al., 1987; Pera et al., 1983). Vesicular-arbuscular-mycorrhiza (VAM) are probably inhibited due to the concentration of phosphorus in the organic amendments, and also as a result of pH buffering by the amendments near pH 7.

Organic Amendments and the Nitrogen Cycle

Due to concerns about groundwater contamination with NO₃-N, studying the several N transformation processes after applications of organic amendments is of critical importance. Figure 2.6 illustrates the different nitrogen pathways and transformations that occur when organic amendments are applied to soil, with most of the transformations

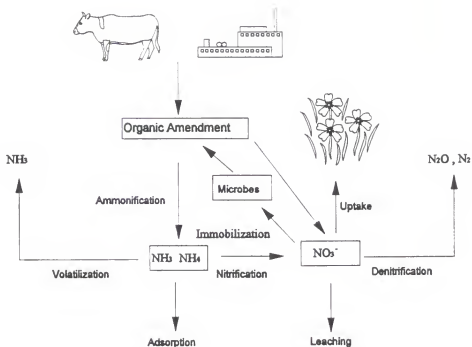


Fig. 2.6 . Fate of nitrogen after application of organic amendments to a soil.

being microbially mediated. As a result, any factor or parameter that regulates microbial growth will also have an effect on the series of N transformations. Nitrogen in organic amendments occurs in both organic and inorganic forms, with the proportion of each depending mainly on the waste-generation process. Typically, sewage sludges contain between 1- 6% N on a dry-weight basis, while MSW composts normally test lower with respect to their N contents.

Nitrogen Mineralization and Immobilization

"Mineralization" is a term used to describe the microbially-mediated process by which organic N is converted to inorganic N, primarily, NO_3^- and NH_4^+ . The rate of N

mineralization in organic-amended soils depends, among other things, on soil moisture, pH, microbial biomass, C:N ratio of the material, and rate and nature of the amendment. It has been commonly assumed that N mineralization rate follows first-order kinetics (Stanford and Smith, 1972). This means that mineralization rate is affected by application rate, as also suggested by Boyle and Paul (1989). The rate appears to be affected as well by the nature of the amendment, as shown by Parker and Sommers (1983). They observed N mineralization of 25% for raw and primary sludge, 40% for waste-activated sludges, 15% for anaerobically digested sludges, and 8% for composted sludges.

Amendments with wide C:N ratios also tend to show slower mineralization rates than amendments with narrower ratios (less than 20:1) as shown by McGill et al. (1981). The pH range over which mineralization takes place has generally been given as 5.5 to 10.0, since this is also the soil pH range for highest bacterial activity. Soil moisture and aeration are important regulators, since *Nitrobacter* are obligate autotrophic aerobes.

Immobilization is the process by which microorganisms use some of the mineralized organic N for their growth and metabolic activities (Alexander, 1977). In reality, however, mineralization and immobilization occur simultaneously, with what is normally measured as "mineralized organic nitrogen" being simply the excess N not used by microorganisms. In the presence of materials with wide C:N ratios (normally larger than 30:1), microorganisms will tend to use most of the N mineralized. In such cases, net mineralization is close to zero. Composted sludges and amendments from paper industries tend to have wide C:N ratios (King, 1984).

Volatilization and Adsorption of Nitrogen

Volatilization of N as ammonia gas (NH_3) occurs at pH values higher than 8.0. King (1973) observed losses of 16 to 22% and 21 to 36% when sewage sludge was incorporated or surface-applied, respectively. More than 50% N loss via NH_3 volatilization can occur rapidly, with 50% of the loss occurring during the first 24 hours (Beauchamp et al., 1982; Donovan and Logan, 1983). Incorporation of the amendments is probably the best way to reduce losses through volatilization. Sims (1992) observed a loss of 10% of the added $\text{NH}_3\text{-N}$ when the material was incorporated immediately, in comparison to a loss of 56% when it was incorporated three days after application. Losses due to volatilization are generally not significant in relation to the total N cycle.

Sludge-borne ammonium also can be adsorbed or "fixed" in the layers of expanding clays such as vermiculite and illite, through a mechanism similar to K^+ fixation. Ammonium may be either permanently "fixed" or eventually be replaced by cations that further expand the clay lattice. Such cations include Ca^{+2} , Mg^{+2} , Na^+ , and H^+ (McBride, 1994).

Leaching of Nitrate

Contamination of groundwater with sludge-borne nitrate is a concern when applying organic amendments to soil, especially under Florida's soil and climatic conditions. When applications of organic amendments are based on the crop's N requirement, such applications do not generally represent a pollution hazard. Maynard (1994) observed no significant difference in $\text{NO}_3\text{-N}$ groundwater concentrations between a control plot and plots receiving MSW compost at equivalent rates of 56, 112, and 224

Mg ha⁻¹. Similar results were obtained by Medalie et al. (1994) after applying up to 14.5 Mg ha⁻¹ sewage sludge to a northern hardwood forest. However, Kingery et al. (1994) concluded that long-term applications of organic wastes at rates higher than the crop nutrient requirement had created the potential for environmental hazard in the Sand Mountain region of Alabama.

Organic Amendments and Denitrification in Soils

Denitrification is the biological conversion of nitrogen oxides, including NO₃⁻ and NO₂⁻, to gaseous N₂ and N₂O. The reaction is carried out by at least 13 bacterial genera (Table 2.7), which use NO₃⁻ or NO₂⁻ as a respiratory electron acceptor and gain energy via coupling to electron-transport phosphorylation (ETP; Tiedje, 1982).

It has been proposed that denitrification proceeds according to the following reactions:



The reactions of denitrification can be defined by competitive Michaelis-Menten-type enzyme kinetics (Cho and Mills, 1979). Nitrate reductase is the enzyme that catalyzes the reduction of NO₃⁻ to NO₂⁻, whereas nitrite reductase catalyzes the reduction of NO₂⁻ to N₂O, and nitrous oxide reductase catalyzes the reduction of N₂O to N₂. All of the NO produced is supposed to be directly reduced to N₂O (Knowles, 1982). These enzymes are not constitutive, and are inhibited in the presence of oxygen. There is also evidence of the existence of non-enzymatic conversion of NO₃⁻ and NO₂⁻ to gaseous forms. Chemo-denitrification occurs under aerobic conditions by various pathways, but is most significant at pH values below 5.0 and in the presence of nitrous acid (Paul and Clark, 1989).

Table 2.7. Genera of bacteria capable of denitrification. (Adapted from Tiedje, 1982)

Genus	Interesting Characteristics of Some Species
<i>Acaligenes</i>	Commonly isolated from soils
<i>Agrobacterium</i>	Some species are plant pathogens
<i>Azospirillum</i>	Capable of N ₂ fixation, commonly associated with grasses
<i>Bacillus</i>	Thermophilic denitrifiers reported
<i>Flavobacterium</i>	Denitrifying species isolated
<i>Halobacterium</i>	Requires high salt concentration for growth
<i>Hyphomicrobium</i>	Grows on one-carbon substrate
<i>Paracoccus</i>	Capable of both lithotrophic and heterotrophic growth
<i>Propionibacterium</i>	Fermentors capable of denitrification
<i>Pseudomonas</i>	Commonly isolated from soils
<i>Rhizobium</i>	Capable of N ₂ fixation in symbiosis with legumes
<i>Rhodopseudomonas</i>	Photosynthetic
<i>Thiobacillus</i>	Generally grow as chemoautotrophs

Denitrification is an important pathway for N transformation, with both economic and environmental implications. Researchers have long studied denitrification in an attempt to reduce loss of plant-available N from agricultural lands. There is also concern that gaseous N forms are contributing to atmospheric ozone depletion (McElroy et al., 1976). In waste treatment plants as well as in soils, denitrification removes excess nitrate that otherwise might end up in the groundwater.

Soil Conditions that Affect the Rate of Denitrification in Soils

There are several conditions that have a primary effect on the rate of denitrification in soils. Tiedje (1988) proposed a conceptual model for denitrification which is based on the hierarchy of the three main regulators of denitrification: oxygen and nitrate concentrations, and organic carbon availability (Fig. 2.7). Based on this model, oxygen is the main regulator of denitrification but oxygen, in turn, is affected by more distal

regulators such as respiration and water content of the soil. The individual effects of each of these factors plus their interaction make denitrification a process that is difficult to either measure or to predict.

Nitrate concentration. Assimilatory nitrate reduction, dissimilatory nitrate reduction to ammonia (DNRA), and denitrification are the microbial processes that use nitrate. The first process is common under aerobic conditions, while the others occur under O_2 -limiting conditions, in the same basic habitat. Under long-term anaerobic conditions DNRA is the major fate of NO_3^- while, in less reduced environments, denitrification may dominate (Tiedje et al., 1981). In flooded soils, NO_3^- diffusion from the overlaying water to the site of denitrification may have an important effect on the rate and order of the reaction (Reddy et al., 1978).

Dendooven and Anderson (1995) showed that, at low NO_3^- concentrations, the K_m (affinity) plays a role in predicting the outcome of competition between denitrification and DNRA but, at high concentrations, the outcome is dominated by V_{max} (related to the density of microbes). DNRA has a higher K_m value and thus must have a ten-fold greater V_{max} than do denitrifiers to process half of the added substrate. According to Paul and Clark (1989), at concentrations above $20 \mu g$ nitrogen ml^{-1} denitrification follows zero-order kinetics. At lower concentrations, Stanford et al. (1975) found that the reaction followed first-order kinetics.

Changes in N_2 and N_2O evolution rates are often associated with changes in the concentration of NO_3^- in soil. Higher concentrations of NO_3^- resulted in increased

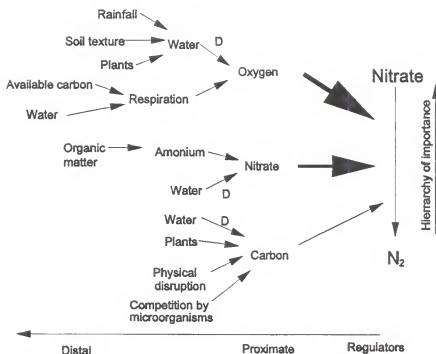


Fig. 2.7. Conceptual model of denitrification. The vertical dimensions show the hierarchy of importance for the three majors regulators of denitrification. The horizontal dimensions show the proximity of the regulatory factors to the process. "D" refers to a diffusion effect on the regulator (Tiedje, 1988).

N_2O production relative to N_2 (Firestone et al., 1979; Weier et al., 1993). This is probably due to the higher affinity of microbes for NO_3^- than for N_2O (Cho, 1982). In a lab study conducted by Dendooven and Anderson (1995) they observed the synthesis of denitrification enzymes to follow a time-dependent order, with nitrate reductase formed within 2-3 hours, nitrite reductase between 4-12 hours, and nitrous oxide reductase between 24 and 42 hours.

Availability of soluble carbon. Denitrification is strongly dependent on a carbon source, since the majority of denitrifiers are heterotrophs. The role of carbon is to provide electron donors for nitrate reduction (Gamble et al., 1977), but denitrifiers respond

differently to different types of carbon sources and to the C:N ratios of the materials (Monteith et al., 1980). For instance, *Alcaligenes* prefer glucose while *Pseudomonas* prefer methanol and ethanol as electron donors. Bijay-Singh et al. (1988) observed that the rate of denitrification was highly correlated to water-soluble organic carbon and less strongly to total organic carbon. Major controllers of available carbon are: a) water, which stimulates metabolism in dry soils and transports available carbon; b) plants, which excrete and deposit carbon; c) physical disruption of protected carbon in the habitat of freezing, thawing, wetting and drying cycles, cultivation or natural disturbances; and d) competition or exclusion by other organisms (Tiedje, 1988).

Some researchers have suggested that easily decomposed carbonaceous compounds exuded by plant roots to the rhizosphere might enhance denitrification, as is the case for corn (*Zea mays*) that excretes into the rhizosphere nearly 25% of the carbon translocated to the roots (Haller and Stolp, 1985). However, such observations are contrary to reports of Guenzi et al. (1978), and Haider et al. (1986), who reasoned that root exudates do not promote N loss since roots compete with denitrifiers for any nitrate present.

Additions of organic carbon through organic amendments may also promote denitrification. King (1973) observed a 20% loss of N by denitrification following surface applications of sewage sludge.

Aeration status of the soil. It is well known that oxygen inhibits the activity, as well as the synthesis, of the enzymes involved in the denitrification process. The mechanisms are still not well understood, but Ferguson (1994) proposed the hypothesis of

an indirect control by oxygen of membrane-bound nitrate reductase activity via a transporter protein. It appears that this membrane-bound nitrate reductase is not inhibited nor inactivated by oxygen, so control of the activity of the enzyme in intact cells must be exerted somewhere other than within the enzyme itself. Several researchers have proposed an oxygen concentration of 0.2 ml l^{-1} as a threshold value below which denitrification is significant (Knowles, 1982). However, this value is qualitative since the threshold concentrations may vary with organism and experimental conditions.

There is also evidence of denitrification occurring in well-drained soils (Bremmer and Blackmer, 1978; Parkin and Robinson, 1989). Some of these studies have shown that typical aerobic denitrification rates represent between 0.3 to 3% of the respective anaerobic rates. Aerobic denitrification rates tend to be highly variable due to the occurrence of "hot spots" of denitrification (Parkin, 1987). Under well-drained conditions, denitrification still might occur around decaying carbonaceous material as long as a supply of carbon exists.

Soil pH and temperature. The majority of denitrifying bacteria are active near neutral pH (6-8; Paul and Clark, 1989), and are not very active below pH 4 or above pH 8 (Bremmer and Shaw, 1958). The $\text{N}_2\text{O}:\text{N}_2$ production ratio is also affected by pH, as observed by Tiedje et al. (1981). These workers reported a considerable increase in N_2O production over that of N_2 when the pH was lowered from 6.7 to 5.2.

Denitrification is an enzymatic process; consequently, temperature also influences the rate of the reaction. However, quantification of the effect of temperature on denitrification is complicated by the fact that temperature has an effect on other N

transformations such as organic N mineralization as well. According to the Arrhenius equation, microbial activity should increase exponentially with increasing temperature. It is commonly assumed that a 10 °C increase in temperature results in a 100% increase in the rate of a biochemical reaction (Q_{10}), though Reddy et al. (1982) reported Q_{10} values between 1.4 and 2.5 for denitrification. Temperature also has an effect on the solubility and diffusion of oxygen. Denitrification is reported to occur between 5 and 75 °C, with the optimum being around 65 °C (Keeney et al., 1979).

Methods to Measure Denitrification

Several methods or techniques have been used to measure denitrification under lab-controlled as well as field conditions. Such methods include the acetylene inhibition technique (Yoshinari and Knowles, 1976), ^{15}N methods (Nommik, 1956), nitrate disappearance, nitrogen balance, and the use of nitrate/chloride ratios (Hauck, 1986). The acetylene blockage technique and the use of isotopes (^{15}N and ^{13}N) are the most commonly used methods to study denitrification.

Acetylene blockage technique. The acetylene blockage technique is based on the inhibition of nitrous oxide reductase, which accumulates in a stoichiometric manner. This principle was first proposed by Fedorova et al. (1973) and confirmed in pure culture by Yoshinari and Knowles (1976). Acetylene (normally 0.1 atm), when used for a limited time, blocks the reduction of N_2O to N_2 . Acetylene also inhibits nitrification; consequently, NO_3^- is not replenished and cannot influence measured denitrification rates.

There are several advantages and disadvantages with the acetylene method. They have been reviewed by Duxbury (1986) and Tiedje (1988). Some of the advantages

include an increase in sensitivity in relation to other methods, use of the natural nitrate substrate pool, a large number of samples that can be analyzed, flexibility of the model for adaptation to different conditions, and relatively low cost when compared to the use of isotopes. Disadvantages of the method include the inhibition of nitrification, degradation of acetylene during prolonged incubation studies, and problems with achieving an even gas distribution in the sample.

The use of nitrogen isotopes. The use of N isotopes to measure denitrification has been common since the 1960s. There are two N isotopes that can be used; the stable isotope ^{15}N and the radioactive ^{13}N . The first one (^{15}N) is the one most commonly used, since the half-life of ^{13}N is less than 10 minutes. Basically, an N balance is done and the unrecovered portion of the isotope is assumed to be the amount denitrified. One of the major advantages of this method is that it allows simultaneous study of other nitrogen transformations such as mineralization and leaching. However, the errors associated with doing an N balance may considerably reduce the effectiveness of the balance method to estimate denitrification rates (Tiedje et al., 1989).

Rolston et al. (1978) proposed a more direct method for field measurements of denitrification by measuring the flux of $^{15}\text{N}_2$ and N_2O from the soil surface. The problem with this approach is that large amounts of ^{15}N -labelled nitrate must be added and rates below $1 \text{ kg of N ha}^{-1} \text{ day}^{-1}$ cannot be detected. Smith (1988) conducted field-based measurements of denitrification by the ^{30}N mass spectrometer method. He measured the $^{30}\text{N}/(^{28}\text{N}+^{29}\text{N})$ ratio, but significant problems were observed in determining the source of the ^{15}N gas produced. Perhaps one of the most important limitations to the use of isotopes

is the high cost associated with the use of this technique.

Detrimental Effects of Organic Amendment Applications to Soils

In addition to nutrients, organic amendments contain variable amounts of trace metals, pathogens and synthetic organics, most of which can become toxic to humans, plants and livestock at given concentrations or numbers. In order to understand the potential health risks associated with land application of organic amendments, knowledge of the exposure pathways for the different toxic compounds is necessary. During preparation of the USEPA's 503 Rule, a pathway approach to risk assessment was used. It consisted of twelve different pathways which may allow the absorption of toxic compounds by humans, livestock, plants, microbes or wildlife (USEPA, 1989).

Plant toxicity from Zn, Cu, Ni, or B resulting from organic amendments is the most limiting pathway for each of these elements, while direct ingestion by humans, livestock, and wildlife is assumed to be the primary limitation for organics, Pb and Fe. Plant uptake and transfer is the main limitation for Cd in the human food chain, and for Mo and Se in the livestock food chain (Chaney and Ryan, 1993). Among the trace metals, Al, Cd, Pb, Mn, and Hg have shown adverse effects on the nervous system (Chang, 1992), while As, Cd, Cr, Pb, Hg, Se and Zn may affect the immune system (Murray and Thomas, 1992). Cadmium, Cu, Pb, and Se produced teratogenic effects in lab experiments (Lewis, 1991), while As, Cd, Pb, Ni, and Cr have shown carcinogenic effects (Burger et al., 1987). Most of the information on the potential risks of land application of organic amendments has been generated for sewage sludge, while research regarding the potential risks of MSW-compost-borne metals is not that abundant.

Excessive applications of organic amendments, at rates larger than plant-N or -P requirements, may subsequently promote the degradation of water reservoirs (eutrophication) or result in groundwater pollution by nitrate. Nitrate is of particular concern in states like Florida, where groundwater is the source of drinking water for up to 90% of the population. Ingestion of high-nitrate waters may cause methemoglobinemia or "blue baby syndrome" in infants less than a year old. Nitrate is reduced to nitrite in the infant's gastrointestinal tract, which in turn oxidizes blood iron in hemoglobin, with methemoglobin being formed (Keeney, 1986). The oxygen-carrying capacity of the blood is consequently reduced. In adults and older children, methemoglobinemia is not a serious problem since the acid levels in the stomachs of such individuals kills most of the bacteria responsible for the reduction of nitrate. High nitrate waters can also become toxic for livestock, though their tolerance level is higher than that of humans.

Pathogenic microorganisms in organic amendments, especially in sewage sludge, are also a concern. Viruses and bacteria represent a risk for groundwater contamination, but that risk is considerably reduced by tertiary treatment that can eliminate up to 90% of the viruses (Asano et al., 1992).

Conclusions From This Literature Review

There is no method of waste disposal or reuse that is 100% risk-free. However, when land applications of organic wastes are practiced following federal and state guidelines or regulations, they present a negligible risk to humans, crops, and the environment as a whole. The Part 503 sludge rule sets criteria for concentrations of ten metals (Chaney, 1989). This rule is based on a risk-assessment approach and on

approximately 20 years of research and practical experience in applying sewage sludge to cropland. The same type of research, but in relation to MSW-compost, is urgently needed.

Concerns are commonly expressed regarding the repeated use of organic wastes - sewage sludge in particular - and the potential for trace metals accumulation in soils and food crops at levels that may be detrimental for human health. These concerns acquire importance since the new USEPA regulations allow metals such as Cr, Cd, Pb, Cu and Ni to accumulate to levels from 10 to 100 times typical background concentrations, and appear to be more permissive than those used by several countries in Europe.

In addition to health concerns, there are also a series of issues that still need to be resolved. Some of those issues include lack of incentives for farmers who use organic amendments, the public's lack of trust in the regulatory agencies, and the potential for liabilities and probable loss of land and crop value.

The use of organic amendments in agriculture has been aggressively promoted in Florida, as has been previously discussed. Additions of organic materials to soils provide carbon, which is the most important electron donor for the denitrification process. Additionally, applications of organic amendments increase the water holding capacity of soils, which in turn promotes anaerobic microsites where denitrification can become a major fate of soil nitrate. Consequently, the effect of such amendments on the rate of denitrification should be the subject of more detailed study. Studies related to the various forms of carbon in organic wastes, and rates of denitrification in waste-amended soils under Florida's unique soil and climatic conditions, are a must.

Denitrification exhibits a high degree of spatial and temporal variability, with coefficients of variation as high as 500%. Existing methodologies may not account for all of the variability but, as long as the limitations and capabilities of each method employed are given consideration, they should still provide valuable information and understanding concerning the process of denitrification.

CHAPTER 3

NUTRIENT AND METALS ACCUMULATION AS A RESULT OF THE LAND APPLICATION OF SEWAGE SLUDGE TO A MATURE CITRUS GROVE

Introduction

The disposal of organic wastes on agricultural lands has been practiced for centuries. However, the increase in the amount of wastes generated, particularly those with higher concentrations and a wider variety of toxic chemicals than 40 or 50 years ago, has resulted in an increased concern for the potential detrimental effects of such wastes on the environment.

The public's opinion regarding land application of organic wastes is mixed. People in larger metropolitan areas, far from agricultural regions, feel more skeptical about the use of wastes in crop production than people living in smaller communities or rural areas. This situation is mainly a consequence of lack of information, and in some cases is due to misinformation and sensationalist journalism.

More than one thousand people immigrate to Florida every day, with most of them relocating to South Florida, and predominantly along the state's southeast coast. This increase in population and consequently in waste generation has prompted studies on the potential impact of waste applications to croplands. The purposes of such studies are

being focussed not only on the generation of necessary data, but also on education of the public regarding this cultural practice.

In 1991, the Palm Beach Soil and Water Conservation District (PBSWCD) initiated a study of the land application of dewatered domestic sewage sludge to a mature citrus grove in Palm Beach County. Aquifer and surface waters were monitored by the South Florida Water Management District (SFWMD) to observe any detrimental effects on water quality as a result of sewage sludge applications. A soil and plant monitoring program was concurrently initiated and conducted by the Soil and Water Science Department of the University of Florida.

The objectives of this study were: (1) to monitor the concentration of nutrients and heavy metals in soil and plant parts for plots that received applications of domestic dewatered sewage sludge; (2) to assess the potential for using sludge as an alternative to chemical fertilizers; and (3) to test for any statistically significant differences in nutrient and metals concentrations among the control and amended plots.

Materials and Methods

The experimental site was located in a mature citrus grove in Palm Beach County, Florida, near the city of Boynton Beach. The grove (29 ha) was divided into four sections for purposes of the study: two western and two eastern blocks. Each block was further divided into 4 - 6 plots, with each plot containing 144 trees. Because of disruption of the control-plot area by a large cypress pond, there was a total of 12 amended plots and 8 control plots. Sludge was applied to the eastern blocks in 3 annual applications of 7-8 Mg

ha⁻¹ each, while the western blocks served as a control. The wastewater residuals, in this case, were obtained from the city of Boca Raton's wastewater treatment plant. This material was stabilized by anaerobic digestion for 15 to 40 days, followed by dewatering to 12% solids using belt filter presses. Sludge was applied March 4 through 14, 1991; April 13 through 23, 1992; and April 10 through 17, 1993.

The northern half of the grove had been planted to citrus of the cultivar Pineapple, while the southern half had been planted to the cultivar Hamlin. Both cultivars were grafted onto sour orange rootstock. The soil in that area belongs to the Myakka series, and is classified as a sandy siliceous hyperthermic Aeric Haplaquod.

Sample Collection

Soil and tissue samples were collected during March and August of each year, while fruit samples were collected at or following harvest in mid-winter. It was initially intended to sample the same trees repetitively throughout the study, but this was not always possible due to a high percentage of tree decline (identified primarily as a virulent strain of tristeza virus).

Soil sampling Soil samples were taken from the drip line of 16 trees from the 0-15, 15-30 and (during the spring sampling only) 30-45 cm depth increments from each of the control and amended plots, using a 2.5 cm (diameter) stainless steel probe. The first sampling event occurred during early fall of 1990, before the first sludge application. Samples were collected in a rotating fashion, with the first sample taken from the drip-line on the south side of a given tree. For the second tree, the sample was taken from the drip-line on its west side. The third soil sample in each block was collected from the drip-line of

the third tree's north side, while the next sample was obtained from the drip-line on the east side of the fourth tree. This pattern was continued for all sixteen trees such that, at the conclusion of each block's sampling, four samples from each of the cardinal points of the drip-line had been collected. At the next sampling period, the sampling points were rotated by 90° for each tree. All sixteen samples were mixed and a 1000 g composite sample was taken from each depth, placed inside a plastic bag, and maintained in an ice chest until it was returned to the lab in Gainesville.

Leaf tissue sampling Tissue samples were taken simultaneously with the soil samples, from the same trees where the soil samples were collected. Five to six leaves from non-fruiting twigs were collected from each tree, for a total of 90 to 100 leaves. Four- to six-month-old leaves were selected for sampling, since the concentration of nutrients reportedly remains fairly stable for leaves of that age. Leaves were placed in a plastic bag and maintained in an ice chest until it was returned to the lab in Gainesville.

Fruit sampling Fruit sampling was conducted in December 1991, March 1993, and January 1994. It was not possible to collect fruit samples from precisely the same trees sampled for leaf analysis for the 1993 and 1994 sampling events, since fruit sampling for these years was conducted after much of the harvest had taken place. A total of 20 fruits were collected from the pre-selected or adjacent trees, discarding any that were abnormal in size, shape and color. Fruits were placed in paper bags and kept cool until they could be delivered to the lab in Gainesville for further processing.

Sample Processing

Upon returning to Gainesville, soil samples were sieved to remove plant roots and other large debris, with a subsample left for immediate analysis of nitrate and pH. The remainder of the soil was air-dried, with a subsample being ground in an agate mortar and pestle for elemental analysis. Samples were stored in plastic containers at room temperature until analysis.

Leaf samples were washed with a P-free detergent (Alconox, Inc. New York), scrubbed lightly with cheesecloth, and then rinsed with deionized water. Samples were air-dried for 24 hours and then placed in a forced air oven set at 60 °C until they achieved constant weight. Dry samples were ground in a Wiley mill equipped with stainless steel blades and screens, and subsequently stored in plastic bottles at room temperature for further analysis.

Fruit samples were also washed with detergent, rinsed with deionized water, and allowed to dry at room temperature. Fruits were cut into halves using a stainless steel knife, with half of the portions being used for further analysis and the rest being discarded. Juice and pulp samples were obtained manually using a kitchen juicer, with the juice being further separated from the pulp by filtration through cheesecloth. The juice was then concentrated by heating a 500 ml subsample on a low-temperature laboratory hot plate until it reached a paste-like consistency. Samples were stored in glass beakers at approximately 4 °C.

Chemical Analysis

Sample digestion. One gram of soil, tissue sample or juice concentrate from each of the composite samples was weighed and digested according to USEPA method 3050 (USEPA, 1982) protocols. This method calls for wet digestion of the sample using nitric acid and hydrogen peroxide. Typically, two days were needed to digest twenty-four samples. Two blanks, two replicates, and certified standards obtained from the National Institute of Standards and Technology (NIST, U.S. Department of Commerce, Gaithersburg, MD) were included with each digestion batch. Two samples were further amended with known concentrations of the certified standard to test the method's recovery and to observe and correct any matrix interferences. Samples were stored in plastic bottles at 4 °C until analysis.

Nutrient and metal analysis. Nutrients were initially analyzed using a Perkin Elmer model 2380 atomic absorption spectrophotometer. Subsequently, some nutrients were also analyzed using a Jarrell Ash inductively coupled argon plasma (ICAP) unit at the University of Florida/IFAS Analytical Research Laboratory. For the analysis of Pb, Cd, Ni, Cu and Zn, the same atomic absorption unit was used but with a graphite furnace attachment. Total-N analysis was performed by Kjeldahl digestion followed by colorimetric analysis (APHA, 1989).

Statistical Analysis

The concentrations of each of the elements were statistically analyzed using SAS PROC GLM (SAS Institute, 1985) to test for significant differences among treatment effects (amended plots vs. control plots), cultivar differences (Hamlin vs Pineapple), depth

of sampling in the case of soil samples, and sampling dates. In addition, interactions among these variables were also measured and tested.

Results and Discussion

The concentrations of metals and nutrients in the sewage sludge used for this study showed a high degree of variation (Table 3.1), with the variability being greater among the trace metals Pb, Ni, and Zn. However, the variability in metal concentration has considerably decreased subsequently, likely due to more stringent regulations. The pH of the sewage sludge was kept near neutrality to reduce the solubility of toxic metals present in the waste.

It is important to note that the grove used for this study was not a highly productive one. In fact, the grove was greatly affected by the tristeza virus, and by micronutrient deficiencies that could have masked some of the more beneficial effects of the sludge. The presence of a cypress pond, several acres in extent, in the control plot planted to the Pineapple cultivar also affected the results.

Concentrations reported in this study represent the total concentrations of the particular elements, including the soil analyses.

Soil Analyses

The concentrations of nutrients and heavy metals in soil from the grove were analyzed for the 0-15, 15-30 and (during spring only) 30-45 cm depth increments during each of the six sampling dates. Differences in P concentrations between the control and the amended plots, and between the Hamlin and Pineapple cultivars, were not statistically

Table 3.1. Elemental analysis of sewage sludge from the city of Boca Raton's wastewater treatment plant for the duration of the study. Analyses were performed by private labs under contract with the local water treatment authority.

	pH	Total N	P	K	Cd	Cu	Pb	Ni	Zn
			----- % -----			----- mg kg ⁻¹ -----			
Feb 91	6.7	11.2	2.20	0.70	3.9	200	17	38	200
May 91	7.2	24.7	4.04	1.20	1.6	157	71	<6	810
Aug 91	6.7	8.3	3.60	8.90	7.4	430	99	45	530
Nov 91	6.8	7.4	1.18	0.90	6.3	680	65	52	933
Jan 92	8.0	4.4	0.19	1.63	1.0	7.7	1.2	2.3	15
Apr 92	7.3	3.0	4.41	0.13	5.8	420	38.5	42	1100
Jul 92	6.9	1.79	3.61	1.65	1.0	8.6	1.4	0.5	15.5
Oct 92	5.8	9.44	4.22	0.63	1.0	9.2	1.0	379	171
Jan 93	7.2	6.22	4.13	1.05	0.5	7.8	10	1.8	17
Apr 93	7.2	1.43	1.11	0.77	2.0	224	17.8	12.9	403
Aug 93	7.3	2.85	1.71	1.21	2.0	230	22	11	570
Dec 93	7.4	4.68	0.43	0.16	1.7	205	16.5	15	383

different ($Pr > F = 0.277$ and 0.3738 respectively), although there was an apparent buildup of P by the end of the trial (Fig. 3.1), specially for the Pineapple cultivar in the amended plots. Soil samples tested consistently higher with respect to P than for the pre-application sampling, likely in response to the grower's fertilization program. Total P concentration

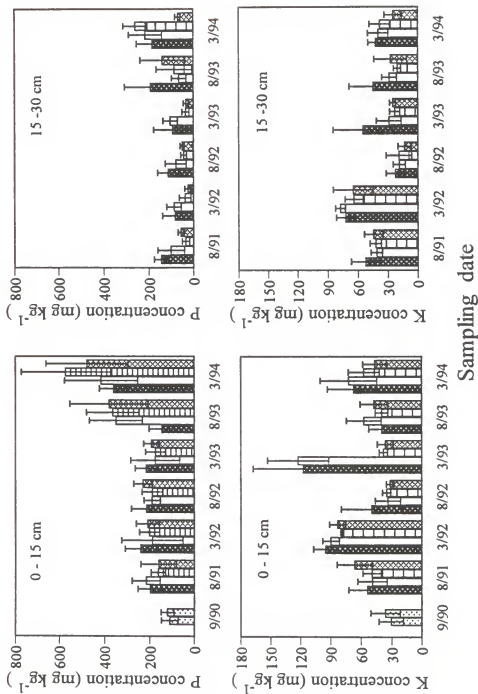


Fig. 3.1. Mean total soil phosphorus and potassium concentrations, and associated standard deviations, for two soil depths. The first two bars for the 0-15 cm increment graphs represent the pre-application period. The first bar starting with August 1991 represents concentrations for the Pineapple cultivar in the control plots, the second bar represents concentrations for the Hamlin cultivar in the control plots, the third represents the Pineapple cultivar in the amended plots, and the fourth bar represents concentrations for the Hamlin cultivar in the amended plots.

also varied significantly between the 0-15 cm and the 15-30 cm depth increments ($Pr > F = 0.0001$), with most of the P remaining in the topmost soil layer.

Concentrations of K proved to be significantly different between depths ($Pr > = 0.0001$) and among control vs. amended plots ($Pr > F = 0.0001$), for the March 1993 sampling. Control plots tended to show higher K concentrations than the amended plots (Fig. 3.1). The higher concentrations observed for the control plots were probably a result of continued chemical fertilizer applications to the control plots. The control and the amended plots were fertilized with 15-0-15 ($N-P_2O_5-K_2O$) material through January 1992, with fertilization continued subsequently only for the control plots. This is the likely reason for the concentration of K being higher during March of each year than at the August sampling time ($P = 0.05$). Even though K is a cation, it could routinely have been leached by the summer rains in addition to plant uptake between March and August-September each year.

The statistical analysis for Ca showed significant differences between treatments ($Pr > F = 0.0005$), sampling dates and sampling depths, but the interactions among these variables were not significant. Calcium concentration in the soil appeared to have increased by the end of the experiment for both varieties and treatment levels (Fig. 3.2), likely as the result of a lime application. Concentration of Mg in the soil followed a trend similar to that for Ca (Fig. 3.2), with the depth and date effects being significantly different from zero ($Pr > F = 0.0001$). However, in this instance, mean Mg concentration in the control plots was not significantly different from mean Mg for the amended plots ($Pr > F =$

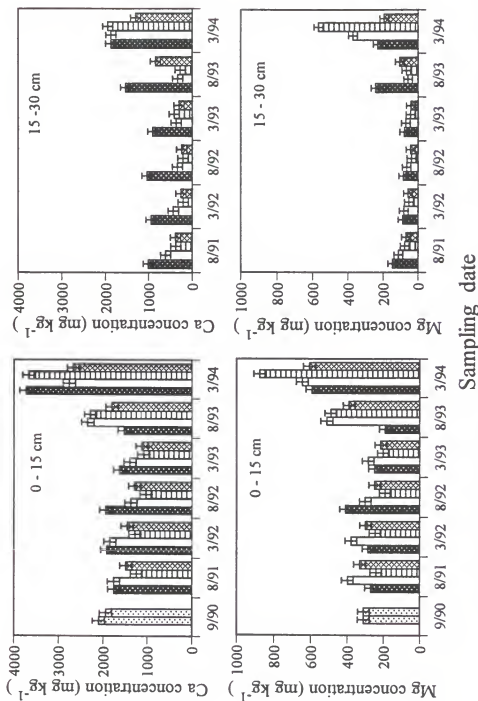


Fig 3.2. Mean total soil calcium and magnesium concentrations, and associated standard deviations, for two soil depths. The first two bars for the 0-15 cm increment graphs represent the pre-application period. The first bar starting with August 1991 represents concentrations for the Pineapple cultivar in the control plots, the second bar represents concentrations for the Hamlin cultivar in the control plots, the third represents the Pineapple cultivar in the amended plots, and the fourth bar represents concentrations for the Hamlin cultivar in the amended plots.

0.4320). Concentrations of both elements in soil were not different from those for the pre-application sampling, for most of the sampling dates until late in the sampling period.

Similar trends to those for the macronutrients were observed for the micronutrients and trace metals. The treatment and cultivar effects were not significant for the Fe concentrations during the present study (Fig.3.3). However, the treatment by cultivar and the treatment by date interactions were significant, specially regarding the Pineapple cultivar during August 1993 and March 1994.

Statistical analysis of the Zn concentration in soil showed no clear trends. However, the concentration of this element in the 15-30 cm increment was approximately half that of the topmost soil layer, for most of the sampling events (Fig. 3.3). Total soil concentration of Cu (Fig. 3.4) in the control plots was significantly higher than in the amended plots ($Pr > F = 0.001$), with this effect being more pronounced with the Pineapple cultivar than with the Hamlin cultivar. The existence of the cypress pond in the "control-Pineapple" block probably contributes to this effect. Moist conditions result in accumulation of organic matter, which in turn contributes to increase in soil cation exchange capacity. Significant differences between the concentration of Cu for the topmost soil layer in comparison to the concentration in the next deeper soil layer were observed. Copper concentrations observed in this study were 10- to 30-fold higher than those reported by Ma et al. (Table 3.2). The concentration of Ni showed a high degree of variability (Fig. 3.4), resulting in significant "cultivar by depth by date" interactions, with the Ni concentrations in the amended plots generally testing higher than the control values at the beginning of the experiment. The average concentration of Ni in the sludge,

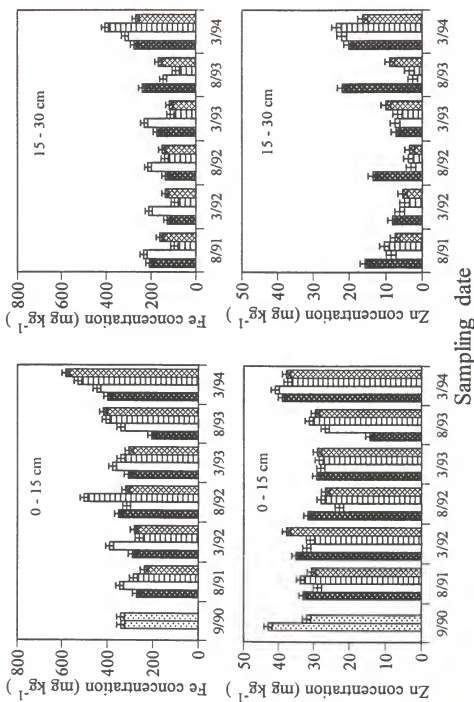


Fig. 3.3. Mean total iron and zinc concentrations, and associated standard deviations, for two soil depths. The first two bars for the 0-15 cm increment graphs represent the pre-application period. The first bar starting with August 1991 represents concentrations for the Pineapple cultivar in the control plots, the second bar represents concentrations for the Hamlin cultivar in the control plots, the third bar represents the Pineapple cultivar in the amended plots, and the fourth bar represents concentrations for the Hamlin cultivar in the amended plots.

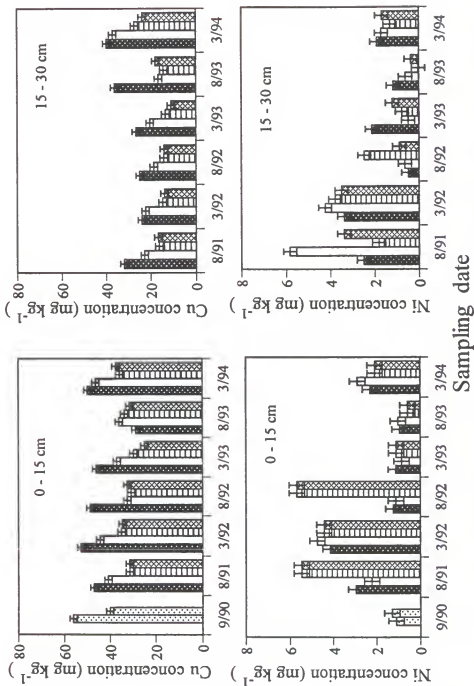


Fig 3.4. Mean total soil copper and nickel concentrations, and associated standard deviations, for two soil depths. The first two bars for the 0-15 cm increment graphs represent the pre-application period. The first bar starting with August 1991 represents concentrations for the Pineapple cultivar in the control plots, the second bar represents concentrations for the Hamlin cultivar in the control plots, the third represents the Pineapple cultivar in the amended plots, and the fourth bar represents concentrations for the Hamlin cultivar in the amended plots.

Table 3.2. Average concentrations for selected metals in the sludge used for this study; in the soil at the study site, previous to the first sludge application; and as reported by Ma et al. (1997) for an unimpacted Florida Spodosol. State guidelines are also provided.

Element	Sludge		Soil	
	This study	Chapter 17-640 [†]	Background [‡]	Ma et al. 1997
	mg kg ⁻¹			
Ni	50.13 ± 105.29 [§]	100	1.0 ± 0.3	6.9
Cu	214.9 ± 208.8	900	43 ± 9.2	1.9
Zn	428.9 ± 370.9	800	38 ± 9.4	10
Pb	30.3 ± 31.9	300	6 ± 0.9	3.2
Cd	2.8 ± 2.4	30	0.3 ± 0.05	0.2

[†] Maximum allowable concentration according to Chapter 17-640 of the Florida Administrative Code.

[‡] Soil analysis during the pre-application period.

[§] Standard deviation of the sample.

by the end of the study, was only one third of that at the beginning of the study. This fact could explain the observed variability with this metal. Despite such variability, values measured in this study (1 - 6 mg Ni kg⁻¹ soil) are close to the value of 6.9 mg Ni kg⁻¹ soil as reported by Ma et al. for a Florida Spodosol. While Cu and Zn are included in small percentages in chemical fertilizers and in larger percentages in some pesticides, Ni is not present in significant quantities in either of those types of chemical formulations. This explains why the concentrations of Ni observed in this study are similar to those reported by Ma et al., with concentrations of Cu and Zn consistently higher.

Soil Cd concentration was not affected by cadmium content of the sewage sludge, since the treatment effect was not significant at the 5% level. Date and depth effects were

significant ($Pr > F = 0.0001$), specially at the beginning of the experiment (Fig. 3.5). A similar trend was observed for Pb concentration in the soil, with the depth and date effects both being significant ($Pr > F = 0.0001$), in part because of higher Pb levels observed during the August 1991 sampling due to higher sludge Pb levels at this time as compared to the sludge used at the end of the study. Concentrations of these two metals were similar to those reported by Ma et al., for the majority of the sampling times.

Leaf Analyses

Diagnostic nutrient concentrations for leaf tissue are presented in Table 3.3. Such ranges were developed for early-fall sampling of the previous spring-flush leaves from non-fruiting twigs. As pointed out previously, until early 1992 chemical fertilizer was applied to both the control and amended plots using a 15-0-15 ($N-P_2O_5-K_2O$) material. According to the grower's records, the amended plots did not receive additional fertilizer applications. Leaf-N concentrations fell within the "optimum" or "high" ranges throughout the study. Nitrogen concentrations in leaf tissue were not different from the concentrations observed during the pre-application period (Fig. 3.6). Statistical analysis of the total N concentrations did not reveal any significant difference between the N concentrations of amended and control plots. Neither was there a significant difference between cultivars; however the "date" effect was significant ($Pr > F = 0.0001$). A series of t-tests showed that the fall leaf samples tested significantly higher than for samples collected during the spring of each year. This observation is in agreement with previous data (Smith, 1966) that showed leaf N concentrations to be more stable during the months of July through October, and to attain the lowest leaf-N contents in the months of January through March.

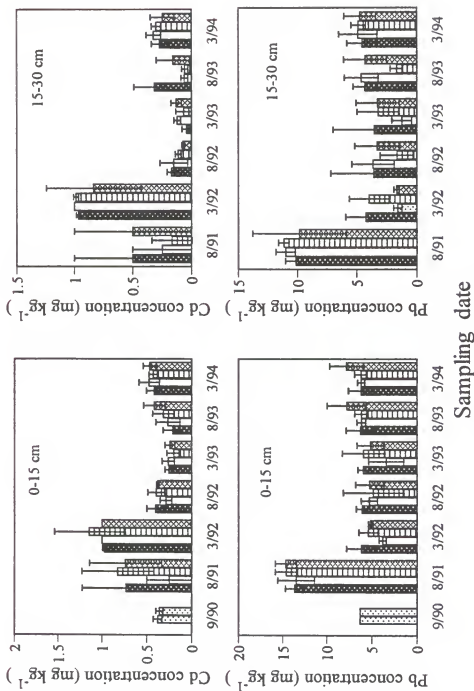


Fig 3.5. Mean total soil lead and cadmium concentrations, and associated standard deviations, for two soil depths. The first two bars for the 0-15 cm increment graphs represent the pre-application period. The first bar starting with August 1991 represents concentrations for the Pineapple cultivar in the control plots, the second bar represents concentrations for the Hamlin cultivar in the control plots, the third represents the Pineapple cultivar in the amended plots, and the fourth bar represents concentrations for the Hamlin cultivar in the amended plots.

Table 3.3 Guidelines for interpretation of leaf analysis for the early fall sampling of 4-6 month-old citrus leaves.

Element	Deficient	Low	Optimum	High	Excess
N (g kg ⁻¹)	< 22	22 - 24	25 - 27	28 - 30	> 30
P (g kg ⁻¹)	< 0.9	0.9 - 1.1	1.2 - 1.6	1.7 - 3.0	> 3.0
K (g kg ⁻¹)	< 7.0	7.0 - 11	12 - 17	18 - 24	> 24
Ca (g kg ⁻¹)	< 15	15 - 29	30 - 49	50 - 70	> 70
Mg (g kg ⁻¹)	< 2.0	2.0 - 2.9	3.0 - 4.9	5.0 - 7.0	> 7.0
Fe (mg kg ⁻¹)	< 35	35 - 59	60 - 120	121 - 200	> 200
Zn (mg kg ⁻¹)	< 17	18 - 24	25 - 100	101 - 300	> 300
Cu (mg kg ⁻¹)	< 3	3.0 - 4.0	5.0 - 16	17 - 20	> 20

Source: Hanlon et al., 1995.

If, in fact, the grower did not apply any chemical fertilizer to the amended plots, the mineralization of organic N present in the sludge (mean = 71.2 ± 63.4 g kg⁻¹) appeared sufficient to maintain the crop's N-requirement.

The "sampling date" effect was the only highly significant effect observed when analyzing the leaf-P data, with the 1991 and 1992 sampling events causing this effect (Fig. 3.6). Except for the March 1992 data, leaf-P concentrations fell within the low range according to University of Florida Special Publication 169 (Hanlon et al., 1995). The effect of sludge-borne P on crop production is influenced by the prior wastewater treatment process. If the sludge is flocculated with iron or aluminum salts, the solubility of P will be reduced considerably. On the other hand, if the sludge is Ca-treated, the solubility of P will not be affected as much, with the resultant material behaving much like

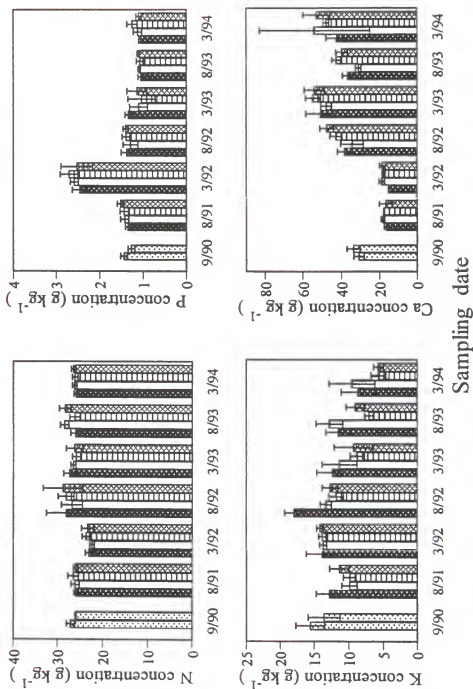


Fig 3.6. Mean leaf tissue nitrogen, phosphorus, potassium, and calcium concentrations, and associated standard deviations. The first two bars represent the pre-application period. The first bar starting with August 1991 represents concentrations for the Pineapple cultivar in the control plots, the second bar represents concentrations for the Hamlin cultivar in the control plots, the third represents the Pineapple cultivar in the amended plots, and the fourth bar represents concentrations for the Hamlin cultivar in the amended plots.

concentrated P fertilizer. Statistical analysis of the K concentrations in leaf tissue showed significant "sampling date" and "treatment" effects ($Pr > F = 0.0001$). The "treatment*date" interaction was also significant, with the t-test showing no difference between treatments (control vs. amended) during 1991 and in March 1992. This coincides with the time when both the control and amended plots received chemical fertilizers. However, the difference in concentration became significant starting in August 1992, when the sludge was the only apparent source of K for the treatment plots. The K concentration in leaf tissue from those plots fell to the deficiency range by the end of the study (Fig. 3.6). The concentration of K in sewage sludge is normally low, no more than 20 g kg^{-1} . The Boca Raton sludge had an average K concentration of $15.0 \pm 21.0 \text{ g kg}^{-1}$ between 1991 and 1993 (Table 3.1).

Leaf Ca concentrations tended to be higher for the amended plots than for the control plots, with the difference being statistically significant during the fall sampling ($Pr > F = 0.0007$). T-tests showed significant differences between treatments for each of the sampling dates except for the August 1991 and March 1992 samples, which tested near the "deficiency" level (Fig. 3.6). The buildup of Ca is probably a result of the sludge being lime-amended as part of the metal-stabilization process at the treatment plant.

Concentrations of leaf Mg fell within the "low" and "optimum" ranges. The Hamlin cultivar showed statistically higher Mg concentrations than the Pineapple cultivar for the amended and control plots, for most of the sampling events (Fig. 3.7).

For Fe, leaf concentration data showed a high degree of variability. There were no significant cultivar nor treatment effects, though the variety*date interaction was

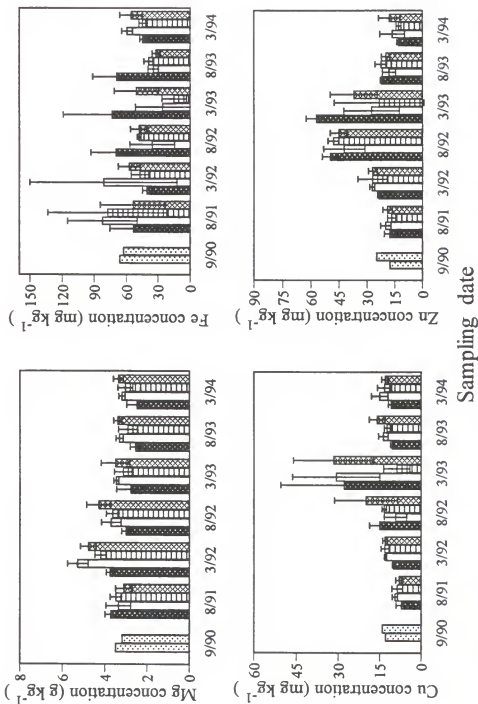


Fig 3.7. Mean leaf tissue magnesium, iron, copper, and zinc concentrations, and associated standard deviations. The first two bars represent the pre-application period. The first bar starting with August 1991 represents concentrations for the Pineapple cultivar in the control plots, the second bar represents concentrations for the Hamlin cultivar in the control plots, the third represents the Pineapple cultivar in the amended plots, and the fourth bar represents concentrations for the Hamlin cultivar in the amended plots.

significant. The concentration of Fe for the amended plots fell near the deficiency limit (Fig. 3.7).

The concentrations of Cu and Zn for the amended plots were not different from their respective concentrations in the control plots. However, while the concentrations of Cu fell within the optimum to high range, that of Zn was typically in the deficiency range (Fig. 3.7). High leaf-Cu concentrations are typical for long-term citrus groves due to the continued use of Cu-based fungicides. Zinc fertilization is normally not recommended, since Zn deficiencies are normally transient in nature at such sites.

Levels of the trace metals Pb and Cd followed similar trends (Fig. 3.8), with Pb and Cd concentrations being significantly higher for the August 1993 sampling than for the rest of the sampling dates. The treatment effect was not significant for either of these trace metals. Although there was no cultivar effect for Pb levels, Cd concentrations tended to be higher for the Pineapple cultivar than for the Hamlin. A t-test showed the difference to be significant only for the August 1992 sampling, however. Nickel concentrations were not significantly different among treatments or citrus cultivars, with sampling date being the only variable that showed a significant effect. There are no guidelines for this set of trace metals in the University of Florida's Special Publication 169 (Hanlon et al., 1995); however, Omran et al. (1988) reported leaf tissue concentrations of 1.11 mg kg^{-1} Pb, 1.76 mg kg^{-1} Cd, and 1.1 mg kg^{-1} Ni in navel oranges after 10 years of wastewater application at unspecified metal concentrations. Furr et al. (1981) reported concentrations of 7.3 mg kg^{-1} Pb, 0.1 mg kg^{-1} Cd, and 0.9 mg kg^{-1} Ni for leaves of sludge-amended Macintosh apples.

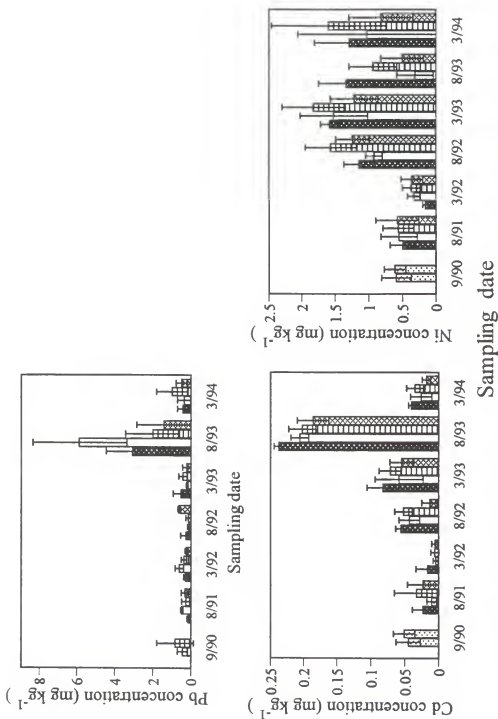


Fig 3.8. Mean leaf tissue lead, cadmium, and nickel concentrations, and associated standard deviations. The first two bars represent the pre-application period. The first bar starting with August 1991 represents concentrations for the Pineapple cultivar in the control plots, the second bar represents concentrations for the Hamlin cultivar in the control plots, the third represents the Pineapple cultivar in the amended plots, and the fourth bar represents concentrations for the Hamlin cultivar in the amended plots.

Juice Analyses

The nutrient and trace metal concentrations in citrus juice may vary depending on the analytical technique being used, as well as on geographical location (McHard et al., 1979). Table 3.4 presents concentration ranges for several elements found in Florida's orange juice along with similar values for Brazilian juice.

Nutrient and metal concentrations reported in this study are based on single-strength orange juice (basically hand-squeezed, pure orange juice). Concentrations of the macronutrients P, K and Ca were two to three times higher than values reported in the literature (Fig. 3.9). In the case of P and K, statistical analysis did not show any significant difference between the control and sludge-amended plots. The only significant effect observed was that due to sampling date ($Pr > F = 0.0001$ and 0.0045 , respectively). In the case of Ca, as for the leaf analysis, concentration of this element in the juice was significantly higher for the amended plots than in the control ones ($Pr > F = 0.0045$). Orange juice of the Hamlin cultivar also showed significantly higher concentrations of Ca than for the Pineapple cultivar. The effect due to sampling date was also highly significant. The "date" effect was the only significant effect observed for Fe, with measured concentrations being in the range of those reported in Table 3.4.

Concentrations of Mg, Cu, Zn and Ni in orange juice were not affected by the application of sewage sludge. The effect of sampling date was statistically significant for Mg, Cu, and Zn ($Pr > F = 0.0001$), but not for Ni ($Pr > F = 0.0959$). The concentration of Mg was three times lower than the typical value, while Cu, Zn and Ni concentrations

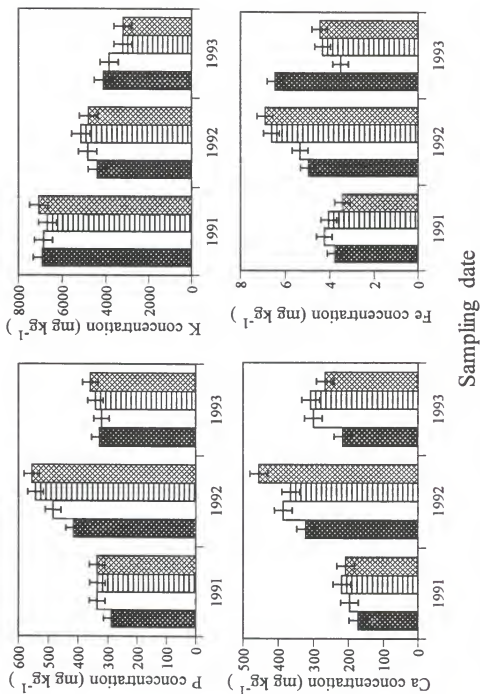


Fig 3.9. Mean juice phosphorus, potassium, calcium, and iron concentrations, and associated standard deviations. The first bar represents concentrations for the Pineapple cultivar in the control plots, the second bar represents concentrations for the Hamlin cultivar in the control plots, the third represents the Pineapple cultivar in the amended plots, and the fourth bar represents concentrations for the Hamlin cultivar in the amended plots.

Table 3.4. Concentration ranges for selected elements in orange juice from Florida and Brazil.

Element	Florida juice	Brazilian juice
----- mg kg ⁻¹ -----		
P	120 - 155 [†]	155 - 310
K	1500 - 1650	2030 - 2400
Ca	65 - 100	80 - 120
Mg	95 - 120	130 - 170
Fe	0.8 - 6.9	0.97 - 17.5
Zn	0.350 - 0.450	0.250 - 0.425
Cu	0.350 - 0.425	0.200 - 0.400
Pb	0.15	0.15
Ni	0.01 - 0.025	0.008 - 0.09
Cd	0.01	0.01

Source: McHard et al. (1979)

[†]Concentrations are based on single-strength orange juice.

were all within the ranges reported by McHard. Concentration of Mg appeared to remain constant, while that of Cu and Zn tended to decrease with time (Fig. 3.10).

Although there was a tendency for the concentration of Pb to increase over the years (Fig 3.11), this trend was not statistically significant. Furthermore, none of the variables appeared to have a significant effect on the concentration of Pb. The Cd concentrations of the orange juice were highly variable (Fig. 3.11), with the control samples testing higher than the amended plots. Juice samples from the Pineapple cultivar tested significantly higher for Cd than for the Hamlin cultivar.

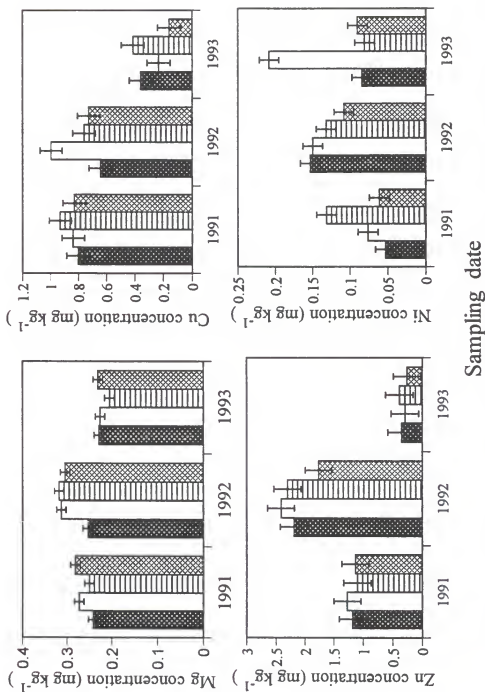


Fig 3.10. Mean juice magnesium, copper, zinc, and nickel concentrations, and associated standard deviations. The first bar represents concentrations for the Pineapple cultivar in the control plots, the second bar represents concentrations for the Hamlin cultivar in the control plots, the third represents the Pineapple cultivar in the amended plots, and the fourth bar represents concentrations for the Hamlin cultivar in the amended plots.

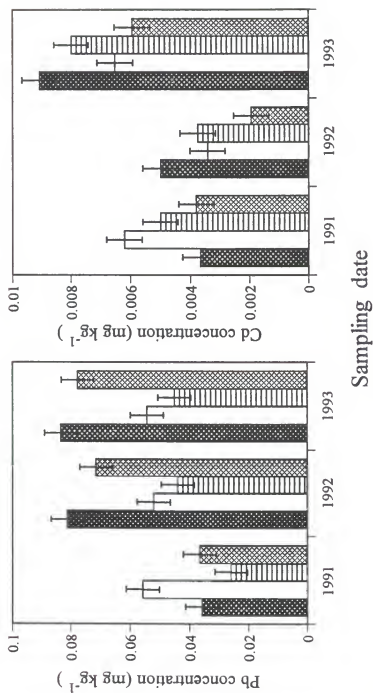


Fig 3.1.1. Mean juice lead and cadmium concentrations, and associated standard deviations. The first bar represents concentrations for the Pineapple cultivar in the control plots, the second bar represents concentrations for the Hamlin cultivar in the control plots, the third represents the Pineapple cultivar in the amended plots, and the fourth bar represents concentrations for the Hamlin cultivar in the amended plots.

Conclusions

The overall objective of this study was to assess the potential detrimental and beneficial effects of land applications of sewage sludge to a sandy soil planted to citrus.

Accumulations of metals were not apparent, due to the low metals concentration of the material in addition to the low application rate employed. Results of this study showed that, of all the nutrients and metals presented here, only soil concentrations of Ca, K, and Cu in the control plots proved to be significantly different from those of the amended plots. Concentrations of K and Cu actually tended to be higher in the control plots, probably due to cultural practices. The "sampling date" and "depth" effects were statistically significant for all elements, with the topmost soil layer testing higher than the 15-30 cm depth for each of the elements analyzed.

Leaf-tissue concentrations for most of the nutrients remained within the normal concentration ranges for citrus leaves. Of the elements in leaf tissue, only the concentrations of Ca and K were significantly different, with Ca concentrations being higher for the amended plots. The K concentration was higher for the control plots and, as assumed before, this difference is probably due to the applications of chemical fertilizer to the control plots and not to the sludge-amended ones. Concentration of Mg in leaf tissue was also significantly different between the control and amended plots, with the Hamlin cultivar apparently accumulating more Mg than the Pineapple cultivar. There were also varietal differences, but only for the concentration of Cu and Cd. However, this difference was evident during the August 1992 sampling period only. There were no clear trends observed for the rest of the elements.

Nutrient and metal concentrations in the juice were not different between the control and amended plots, with the "sampling date" effect being the only significant response. The variability due to sampling date may have several causes. One reason may be that fruit samples were taken at different times during each of the three years that fruit samples were collected. Another reason might involve normal nutrient cycling during the year, with nutrient concentrations being more stable during the months of August through October. Lastly, the variable age and health conditions of the grove probably influenced concentrations of the elements analyzed, due to different sink-source relationships and root morphology among newly planted and established trees.

Based on the chemical and statistical analysis of this study, sludge applications of 7-8 Mg ha⁻¹ per year to a mature citrus grove did not appear to produce an increase in the concentrations of heavy metals in soils nor in associated plant parts. This is specially of concern for elements such as Cd and Pb. The sludge used in this study apparently supplied a sizeable portion of the N and P crop nutrient requirements. In consequence, the value of sludge as a source of nutrients for plants should also be acknowledged.

Although results of the present study showed no significant effects due to the application of sewage sludge to cropland, there are still some critical questions that need to be resolved. The long-term effects of sludge applications under Florida's climatic and soil conditions, the differences in uptake among plant species commonly grown in the state, and multi-element toxicities are only a few of the areas that need to be addressed by scientists subsequently.

CHAPTER 4 REGULATION OF DENITRIFICATION IN A SANDY SOIL

Introduction

The reduction of nitrate to N_2O and N_2 in a given soil is regulated by the numbers and potential activity of existing denitrifiers, in addition to several environmental factors. The most important environmental factors that regulate denitrification include aeration status of the soil, nitrate concentration, the presence and concentration of a soluble energy source, and temperature.

It is known that oxygen inhibits both denitrifying enzyme activity and the synthesis of new enzymes involved in the denitrification process. Oxygen diffusion rates are four orders of magnitude lower in water than in the gas phase; for this reason, oxygen concentration at the microbial cell is regulated by the water content of the soil. Denitrification occurs predominantly in soils that are under water-saturated or near-saturated conditions. The point at which all or most of the soil porosity is occupied with water will vary among soils, and is predominantly regulated by soil particle-size distribution. Under well-aerated conditions, denitrification still proceeds but only at the microsite scale, probably around decaying organic matter or inside anaerobic soil aggregates.

Denitrification is a process that follows zero-order kinetics with respect to nitrate under non-limiting concentrations such as those occurring in most agricultural soils. The process follows first-order kinetics under nitrate-limiting conditions such as encountered in most natural habitats.

Denitrification is strongly dependent on the presence of a suitable carbon source (electron donor), since most denitrifiers are heterotrophs. Soluble carbon is normally the main regulator of denitrification in groundwater. Numerous studies have shown that additions of a carbon source result in a considerable increase in the rate of denitrification for agricultural subsoils, down to their associated water tables.

Temperature is the other factor that plays an important role in the regulation of denitrification, specially since the process is enzymatically mediated. The effect of temperature on denitrification is of particular importance for the present study, since soil samples were collected for study from tomato beds that are covered with black plastic mulch. The practice of using plastic mulch results in higher soil temperatures than for corresponding bare soil.

Objectives of the present study are three. Objective one is to observe how variations in the percent water-filled pore space (WFPS) affect the rate of denitrification for soils amended with MSW and for unamended soils. It also includes the definition of an empirical relationship between percent WFPS and denitrification rate. Objective two includes studying the effect of varying concentrations of electron donors and electron acceptors on the rate of denitrification. Objective three involves studying the temperature

dependance of denitrification for MSW-amended and unamended soils covered with plastic mulch.

Materials and Methods

Soil Collection and Handling

Bulk samples of surface soil (0 to 20 cm) were obtained from an Eugaillie fine sand (sandy, siliceous, hyperthermic Alfic Haplaquods) planted to tomato, amended with approximately 30 Mg ha⁻¹ municipal solid waste (MSW) compost and also from a bed that had not received any organic amendments. The samples were collected at the University of Florida's Gulf Coast Research and Education Center, in Bradenton from tomato beds irrigated by maintaining a perched water table 45 cm deep. Samples were kept in an ice chest and transported to Gainesville, where they were stored under field-moist conditions at 4 °C until used.

Denitrification Measurements and Data Analysis

The acetylene blockage technique (Yoshinari and Knowles, 1976) was used during all studies to estimate the rate of denitrification in the soil under study. The acetylene used for the studies was purified by a two-step washing technique (Hyman and Arp, 1987). First, the gas was washed in concentrated sulfuric acid to remove acetone and phosphine. Acetone is an antibiotic, and phosphine is a highly toxic compound. Then, any sulfuric acid remaining in the acetylene was removed by washing the gas with 5 N sodium hydroxide. The acetylene was washed before each experiment and kept in gas bags.

Gas samples were collected periodically and stored in preevacuated vials (Becton Dickinson, USA) for subsequent analysis. Nitrous oxide was analyzed on a Shimadzu

14-A gas chromatograph equipped with a ^{63}N electron capture detector (ECD), and fitted with a packed 3.2 mm by 1.8 m stainless steel column (Porapak Q). Operating conditions included: column temperature, 30 °C; ECD temperature 300 °C; injector temperature 110 °C; and 5% CH_4 / 95% argon carrier gas. Commercially available standards were used to calculate the nitrous oxide concentration in each vial.

Chromatograph peaks were transformed into gas concentrations. Nitrous oxide data then were plotted against time and a regression equation was fitted using EXECUSTAT (EXECUSTAT, 1991) to calculate rates of denitrification. Data were checked for normality, and the Durbin-Watson parameter was used to check for any interdependence of errors.

The amount of N_2O dissolved in water was calculated using the Bunsen absorption coefficient, according to the relationship (Tiedje, 1982)

$$M = C_g (V_g + V_l \alpha),$$

where M = amount of N_2O in the water plus gas phases, C_g = concentration of N_2O in the gas phase, V_g = volume of the gas phase, V_l = volume of the liquid phase, and α = the Bunsen absorption coefficient.

All incubations were conducted in the dark in an orbital incubator shaking at approximately 100 rpm, to avoid diffusion effects. Four mL of the headspace gasses were collected periodically through the stopcock and stored in preevacuated vials for subsequent analysis.

Effect of Temperature on Denitrification

Subsamples, 100 g each, of the unamended and amended soil were weighed and placed in triplicate into 125 ml glass serum bottles (Cole Palmer, USA) for each of the selected temperatures (25, 35, and 45 °C). Then, 100 mL of a solution containing 5 mM KNO_3 and 5 mM glucose were added to each bottle, followed by acetylene equivalent to 15% of the headspace. Serum bottles were tightly capped and a syringe needle was attached to a two-way airtight stopcock (Cole Palmer, USA) and passed through the cap. A similar type of experiment, normally called measurement of the denitrifying enzyme activity (DEA), was also conducted. This test consists of incubating soil samples under non-limiting conditions. Chloramphenicol is added to each bottle to inhibit the formation of new enzymes for a short time, typically 2 hours. The use of this inhibitor is useful to test the response of existing denitrifiers to sudden changes in temperature.

The dependence of the rate of denitrification on absolute temperature is accounted for by the Arrhenius equation as derived from thermodynamic considerations:

$$\frac{d(\ln K)}{dT} = \frac{Ea}{RT^2} \quad \text{or} \quad K = A \exp \left(\frac{-Ea}{RT} \right)$$

Integration with respect to T gives

$$\ln K = \ln A - \frac{Ea}{RT}$$

where

K = rate of denitrification,

A = a constant that is independent of temperature for a given reaction,

Ea = activation energy,

R = universal gas constant (8.34 J mole⁻¹ °K), and

T = absolute temperature (°K).

Q₁₀ was calculated as :

$$Q_{10} = \exp^{\left(\frac{10E_a}{RT^2}\right)}$$

Effect of Varying Water-Filled Pore Space (WFPS) on the Rate of Denitrification

Percent water-filled pore space is probably a more practical index of soil aeration than percent soil water holding capacity (WHC), since it requires only a knowledge of gravimetric soil water content and soil bulk density. Percent WFPS can be calculated based on the relationship:

$$\text{Soil porosity (\%)} = \left(1 - \frac{\rho_b}{\rho_s}\right) \cdot 100 ,$$

assuming a soil particle density (ρ_s) of 2.65 Mg m⁻³. Soil bulk density was measured using a bulk density kit.

Fifty grams of Eaugallie fine sand soil amended with compost at an equivalent rate of 30 Mg ha⁻¹, and of unamended soil, were weighed and placed in triplicate into 125 ml serum bottles, for a total of 24 bottles. Then, solutions containing 150 mg glucose-C kg⁻¹ soil, and 500 mg KNO₃-N kg⁻¹ soil, were added to each bottle to attain WFPS values of 40, 60, 80, and 100%. Acetylene equivalent to 15% of the headspace was added to each bottle using a disposable syringe. Serum bottles were then incubated in an orbital

incubator shaking at approximately 100 rpm. Four mL of the headspace gasses were collected periodically through a stopcock and stored in preevacuated vials (Becton Dickinson, USA) for subsequent analysis. Samples were analyzed for nitrous oxide as described previously.

The Effect of Varying the Concentration of Glucose-C and KNO_3 -N on the Rate of Denitrification.

An incubation study was conducted to observe the effect of varying concentrations of electron donor (glucose) and electron acceptor (nitrate) on the rate of denitrification in a sandy soil. Fifty grams of soil were weighed into 125 ml glass serum bottles, for a total of 27 bottles. Fifty mL of solution were then added to each bottle to attain glucose-C concentrations of 0, 150, and 300 mg kg^{-1} soil, and KNO_3 -N concentrations of 50, 150, and 250 mg kg^{-1} soil. Acetylene equivalent to 15% of the headspace was added to each bottle. Bottles were incubated at room temperature and were constantly shaken, typically at 180 strokes per minute to assure good gas and solution distribution. Four mL of the headspace gasses were collected periodically through a stopcock and stored in preevacuated vials for subsequent analysis. Analyses for nitrous oxide were done as described previously.

Results and Discussion

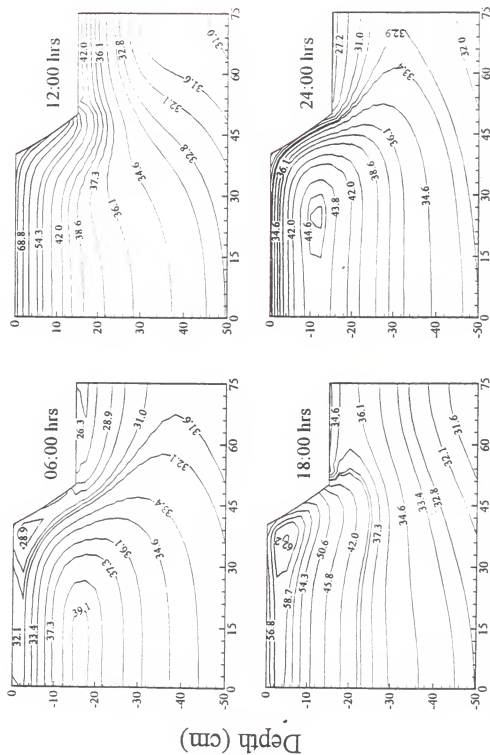
The Effect of Temperature on the Rate of Denitrification

Computer simulations. Figures 4.1 and 4.2 show computer simulations of temperature in portions of an Eaughallie fine sand which has been formed into a crop-production bed and covered with black plastic mulch. Simulations were performed using a

model of coupled water, heat and solute transport for mulched soil-bed systems (Shinde et al., 1996). The model simulates soil temperatures for a plastic-mulched bed without including the shading effect of the crop, in this case tomato. Such assumptions will result in an overestimation of the simulated temperatures since, with tomato, nearly 100% of the bed will be shaded by the plant canopy by the seventh week after transplanting.

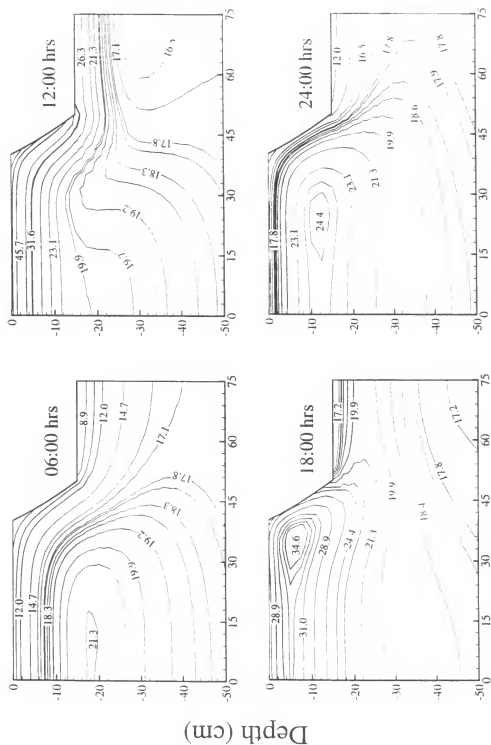
Figure 4.1 shows the simulated soil temperature distribution for a day with an average air temperature of 29 °C, at four times during the day (6 am, 12 noon, 6 pm, and 12 midnight). Simulated temperature values agree with published results under similar conditions (Albregts et al., 1996; Ham and Kluitenberg, 1994). Temperature fluctuations are pronounced in the top 30 cm of soil, though daily temperature remains fairly constant at greater depths. Simulated temperatures adjacent to the plastic mulch reached 32 °C at 6 am but, by noon, temperatures had reached the 72 °C mark. These temperatures tended to decrease by 20 and 40 °C by 6 pm and midnight, respectively. Although simulated temperatures were as high as 72 °C in the top soil layer, they were 30 °C lower only 10 cm into the soil profile. Simulated temperatures around the area not covered with plastic mulch were considerably lower than for those areas which were covered, and showed more gradual fluctuations.

Figure 4.2 gives the simulated temperature distributions for a day with an average air temperature of 18 °C. Temperature profiles followed the same trends as for the warmer day, but fluctuations were evident only in the top 10 cm of depth. The highest temperatures simulated under the specified conditions were observed at noon, beneath the plastic mulch. However, 10 cm into the soil profile, the temperature was only half of that



Distance from bed center (cm)

Fig.4.1. Temperature simulations throughout the soil profile of an Eugallie fine sand covered with plastic mulch, with a water table 45 cm deep. Average air temperature was set at 29 °C.



Distance from bed center (cm)

Fig. 4.2. Temperature simulations throughout the soil profile of an Eauggallie fine sand covered with plastic mulch, with a water table 45 cm deep. Average air temperature was set at 18 °C.

at the surface. Temperature in the subsoil oscillated between 18 and 24 °C, and remained relatively constant.

Diurnal fluctuations in soil temperature by 10 or 20 °C, as predicted by the simulation model, can influence the rate of denitrification, since denitrification is an enzyme-mediated process. If that is the case, denitrification may be inhibited at low temperatures, during the early mornings of cold days with temperatures of 10 °C or less, and during the afternoon of warmer days with soil temperatures near 70 °C. In each case, minimum, optimum, and maximum temperatures for nitrate reduction will vary among microbial species and strains, and with environmental conditions (Reddy and Burgoon, 1997).

Lab incubations. Soil samples amended with MSW or glucose-C were incubated at 25, 35 and 45 °C for a period of five hours as described previously. The production of nitrous oxide over this time period is presented in Fig. 4.3. A linear trend was observed between incubation length and gas production for all treatments at each of the selected temperatures.

Calculated denitrification rates ($\text{ng N}_2\text{O-N g}^{-1} \text{ h}^{-1}$) for the 5 hr incubations were significantly higher ($p < 0.05$) than rates for the DEA (2 hr incubations) for both the compost- and glucose-amended samples (Table 4.1). Denitrification rates for the control samples ($\text{KNO}_3\text{-N}$ amended, but no carbon added) were significantly lower than rates for the rest of the treatments, at any temperature. Denitrification rate for the compost-amended samples at 25 °C was nearly three times that for the glucose-amended samples,

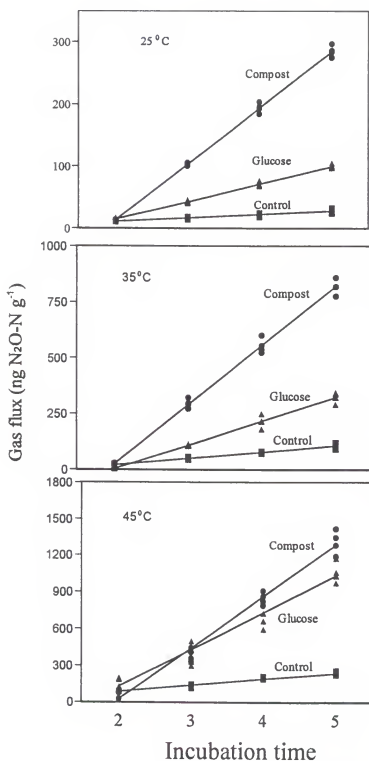


Fig. 4.3. Gas production from samples incubated under different temperatures and energy sources. The energy sources were MSW-compost at a rate equivalent to 30 Mg ha⁻¹, glucose-C, and soil organic carbon (control). Bars represent ± 1 standard deviation.

Table 4.1. Denitrification rates calculated at different temperatures for compost-amended, glucose-amended and unamended (control) surface soil samples.

Treatment	Temp.	Rate		R ²	
		5 hrs	DEA [†]	5 hrs	DEA
	(° C)	----- ng N ₂ O-N g ⁻¹ h ⁻¹ -----			
Control	25	5.65 (0.69)		0.87	
	35	28.68 (3.91)		0.84	
	45	51.44 (3.54)		0.95	
Compost	25	90.16 (4.19)	15.61 (1.45)	0.97	0.92
	35	264.60 (8.99)	57.3 (4.78)	0.98	0.93
	45	416.8 (21.79)	167.3 (9.54)	0.97	0.97
Glucose	25	28.22 (1.75)	4.49 (0.4)	0.97	0.92
	35	106.65 (10.89)	5.79 (0.42)	0.91	0.95
	45	298.9 (31.12)	8.76 (1.58)	0.90	0.75

[†]Denitrifying enzyme activity.

Numbers in parentheses represent standard errors of the corresponding rates.

All slopes are significant at the 5% level (n = 15).

and 18 times that of the control treatment. Denitrification rate for the compost-amended samples at 35 °C was nine times higher than for the control, and twice that at 45 °C.

Carbon-amended samples, whether glucose-C or compost-C, showed higher gas fluxes than the control samples over the length of the study. This is probably the result of an increase in denitrifier biomass with increase in soluble organic carbon concentrations

for these samples, since most of the denitrifiers in soil are chemoheterotrophs. On an equivalent soluble organic carbon basis, compost-amended samples had four times more soluble organic carbon than glucose-amended samples. This is the most likely reason for the higher denitrification rates observed for the compost-amended samples in relation to the glucose-amended samples.

The temperature dependence of a reaction or process has traditionally been characterized with the Arrhenius equation. Figure 4.4 presents Arrhenius plots which describe the relationship between natural log of the rate and reciprocal of the absolute temperature. Using this graph, Q_{10} values (the factor by which denitrification rate increases when temperature is raised by 10 °C) of 3.0, 2.2, and 3.1 were calculated for the control, compost-amended, and glucose-amended samples, respectively. Shown in Fig. 4.4 as well is an Arrhenius plot for the DEA rates. Calculated Q_{10} values for DEA were 1.3 and 3.3, respectively, for the glucose-amended and compost-amended samples. Although linear regression equations were fitted to the measured rates, the data appeared to follow a "two-step" trend, with a larger increase in rate between 25 and 35 °C than between 35 and 45 °C.

Average temperatures at the Gulf Coast Research Center (Fig. 4.5) have oscillated for the last 40 years between 15 °C (November through February) and 28 °C (May through September). These ranges of temperatures should regulate the denitrification process, as discussed here.

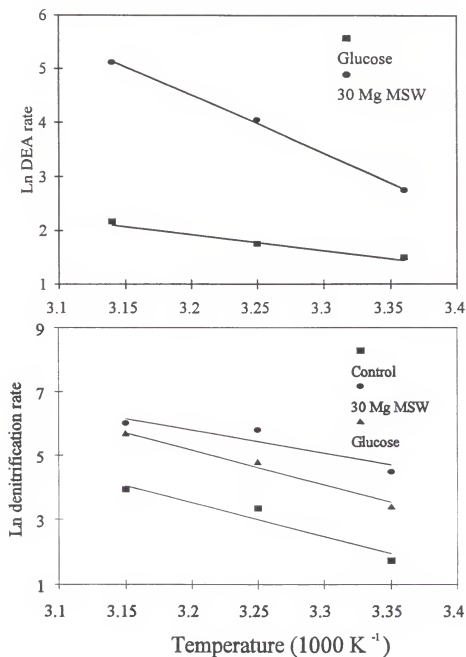


Fig. 4.4. Arrhenius plots of denitrification rates for samples incubated under different conditions, and the associated regression lines. The top graph represents DEA rates (2 hrs incubation). The bottom graph is total denitrification (5 hrs incubation).

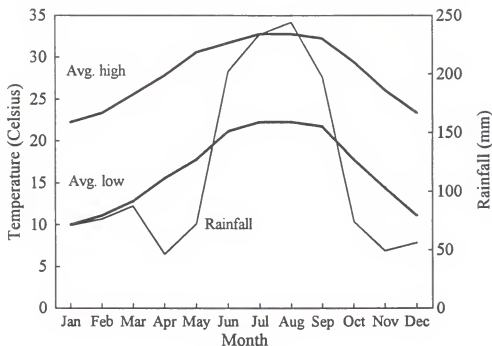


Fig. 4.5. Overview of the average temperatures and rainfall for Bradenton, Florida for the last 40 years.

The Effect of Varying Water-Filled Pore Spaces on the Rate of Denitrification

The production of nitrous oxide gas increased with increasing percent WFPS for both the control (non-amended) and the MSW compost-amended samples (Fig. 4.6). Production followed a linear trend with respect to incubation time for both treatments, though there was limited gas production below 60% WFPS for the control treatment. This value corresponds to approximately 22% volumetric water content, and is in general

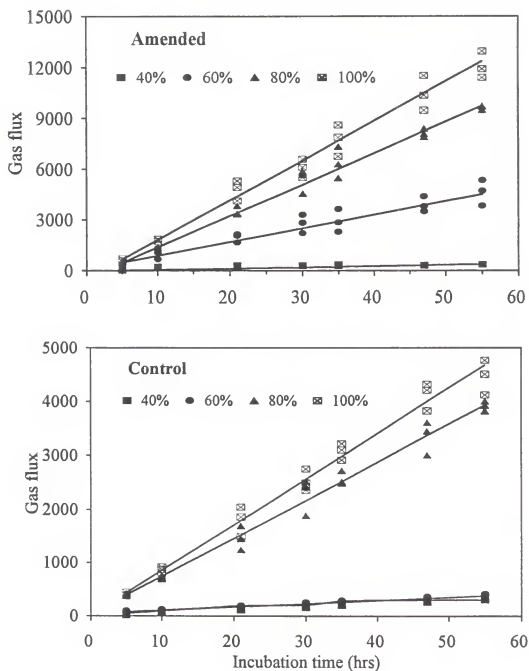


Fig. 4.6. Nitrous oxide production for a soil incubated under varying percent WFPS. The top graph represents a soil amended with an equivalent rate of 30 Mg ha⁻¹ MSW-compost. The bottom graph represents an unamended soil. Gas flux is given as ng N₂O-N g⁻¹ soil.

agreement with results from previous studies (Aulakh et al., 1982; Linn and Doran, 1984) that have shown that denitrification becomes an important pathway for nitrate at WFPS larger than 60%. Gas production by the amended samples was limited below 40% WFPS, find studies where organic-amended soils were related to percent WFPS and denitrification, however. Weier et al. (1993) incubated samples amended with glucose-C and $\text{KNO}_3\text{-N}$ and found values following the same trend as observed in this study.

The calculated rate of denitrification, standard errors for the estimates of the rates, and R-squared values are shown in Table 4.2. The p-value for the lack-of-fit test was non-significant at the 5% confidence level for the treatments, meaning that a linear model represents the data adequately. Calculated rates for the control treatment at 40 and 60% WFPS were not significantly different from one another at the 5% confidence level (5.54 vs. 6.19). Those rates are significantly different from the denitrification rates for the control samples at 80 and 100% WFPS, however.

Denitrification rates at 80 and 100% WFPS were between 10 and 15 times larger than those calculated at 40 and 60% WFPS. The rate for the amended samples at 100% WFPS was 1.2, 3, and 33 times larger than the rates at 80, 60 and 40% WFPS, respectively.

Denitrification rates for the amended samples were higher than those calculated for the control treatments under equal WFPS conditions. The reason for this difference is that additions of a carbon source tend to promote microbial growth, as well as the occurrence of anaerobic microsites. Anaerobism may also be induced as a result of the

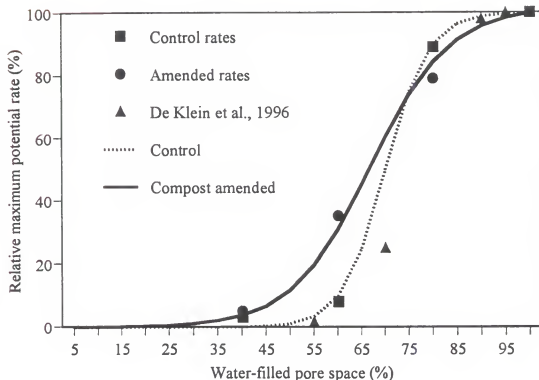
Table 4.2. Denitrification rates calculated for samples incubated under different moisture conditions. Samples included soil which was MSW compost-amended at a rate of 30 Mg ha⁻¹, as well as unamended soil samples. All slopes are significant at the 5% level.

Treatment	WFPS	Rate	R ²
	(%)	(ng N ₂ O-N g ⁻¹ h ⁻¹)	
Control	40	5.54 (0.93) [‡]	0.70
	60	6.19 (1.05)	0.69
	80	70.80 (6.89)	0.86
	100	84.88 (5.24)	0.90
Amended	40	7.21 (0.28)	0.97
	60	81.07 (6.36)	0.89
	80	185.18 (6.23)	0.97
	100	233.47 (15.58)	0.92

[‡] Numbers in parentheses are standard errors of the rates.

increased water holding capacity provided by the organic amendment, along with an increase in the rate of respiration (oxygen consumption).

A non-linear relationship between water-filled pore space and relative change in the rate of denitrification is presented in Fig. 4.7. A logistic model was fitted using SAS-NLIN procedure (SAS Inc., 1985). Based on this graph, denitrification rates sharply increase at WFPS values above 60% (22% volumetric water content) for the control samples ($R^2 = 0.96$), and reach 50% of the maximum rate at 70% WFPS. For the amended samples ($R^2 = 0.98$), denitrification increased at WFPS values above 45%



$$Control = \frac{100}{1 + 99 * \exp(-0.19 * (WFPS - 46))}$$

$$Amended = \frac{50}{0.5 + 99.5 * \exp(-0.13 * (WFPS - 25))}$$

Fig. 4.7. Relationship between percent water-filled pore space and relative maximum potential rate of denitrification, and associated parameter values for the fitted models. The solid line represents samples that were amended with an equivalent rate of 30 Mg ha⁻¹ MSW-compost. The dotted line represents samples that received no amendments.

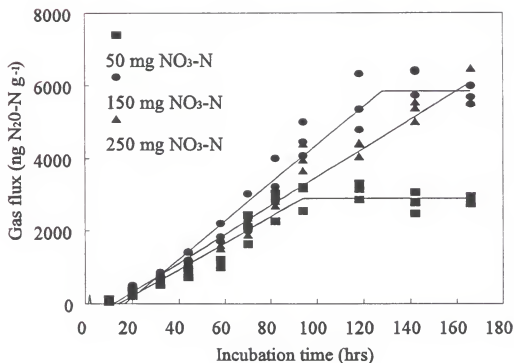
(16% volumetric water content), reaching 50% of the maximum rate at a WFPS value of approximately 65%.

A relationship of this type may be even more appropriate for predictive purposes than a relationship using the measured rates. The measured rates likely vary depending on specific soil conditions at the time of sampling, while the relative change in rate with percent WFPS will tend to remain constant, regardless of the magnitude of the calculated rate.

The fact that the threshold value for the amended samples is lower than that for the control samples may be related to a higher water holding capacity per unit area, in addition to higher electron-donor pressure in such samples. Both conditions allow the development of anaerobic microsites and, consequently, increase the denitrifying activity.

The Effect of Varying the Concentration of Glucose-C and $\text{KNO}_3\text{-N}$ on the Rate of Denitrification

The soil used for this study had an average water-soluble organic carbon concentration of approximately 100 mg kg^{-1} soil. However, the nature of this form of carbon is not known. Figure 4.8 is a plot of $\text{N}_2\text{O-N}$ production over time for samples that received additions of $\text{KNO}_3\text{-N}$ equivalent to 50, 150 and 250 mg kg^{-1} soil. Glucose-C was not added in this case. A linear plateau model was fit to the data using the SAS-NLIN procedure (SAS Institute, 1985). The slope for the linear portion of the $50 \text{ mg KNO}_3\text{-N kg}^{-1}$ soil treatment was $36 \text{ mg NO}_3\text{-N kg}^{-1} \text{ soil hr}^{-1}$, with the data reaching a plateau or critical point (CP) after 94 hours of incubation. The plateau is the point at which the concentration of nitrate-N falls below the minimum concentration required to sustain



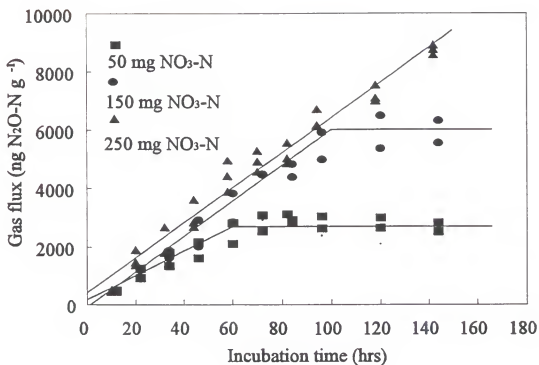
	50 mg KNO ₃ -N kg ⁻¹	150 mg KNO ₃ -N kg ⁻¹	250 mg KNO ₃ -N kg ⁻¹
Slope	36.19 (2.75)	52.59 (3.01)	39.53 (1.19)
Critical Point	93.99 (4.81)	128.73 (6.05)	
Intercept	-511.08 (140.48)	-883.85 (203.42)	-470 (107.05)
R ²	0.93	0.95	0.97

Fig. 4.8. Nitrous oxide production over time, associated regression lines, and model parameters for samples incubated with 50, 150, and 250 mg KNO₃-N kg⁻¹ soil. No glucose-C was added. Numbers in parentheses are standard errors of the estimates.

denitrification. The slope for the 150 mg $\text{KNO}_3\text{-N kg}^{-1}$ soil treatment was 53 mg $\text{NO}_3\text{-N kg}^{-1}$ soil hr^{-1} and reached a plateau after 128 hours, 34 hours longer than for the previous treatment. For the 250 mg $\text{KNO}_3\text{-N kg}^{-1}$ soil treatment, a linear regression equation was fitted, since the plateau (CP) was not observed during the total length of the study (166 hours). The calculated slope was 39.53 mg $\text{NO}_3\text{-N kg}^{-1}$ soil hr^{-1} .

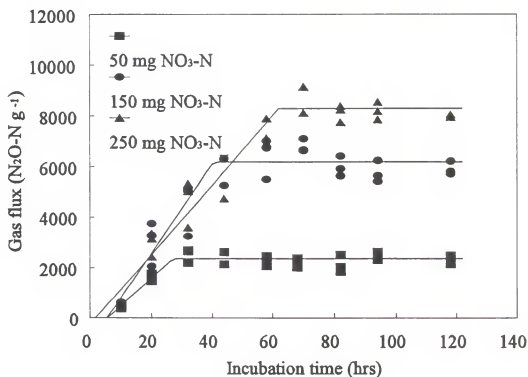
Figure 4.9 is also a plot of $\text{N}_2\text{O-N}$ flux over time. However, an additional 150 mg glucose-C kg^{-1} soil were added to each bottle. All slopes and critical points are significantly different from the 0 glucose-carbon (control) treatment. Calculated slopes were 42 (14% higher), 61 (14% higher), and 60 (34% higher treatment) than the "no-glucose-C" treatment for the 50, 150 and 250 mg $\text{KNO}_3\text{-N kg}^{-1}$ soil treatments, respectively. The observed critical points at which the gas flux reached a plateau for the 50 and 150 mg $\text{KNO}_3\text{-N kg}^{-1}$ soil treatments occurred 34 and 28 hours sooner than for the previous treatment (0 glucose-C). Such critical points occurred 60 and 100 hours after initiation of the experiment. For the 250 mg $\text{KNO}_3\text{-N kg}^{-1}$ soil treatment, a linear regression equation was fitted to the data since a critical point was not observed during the length of the experiment.

The same type of information as in the previous graphs is presented in Figure 4.10 as well, but only after an additional 300 mg glucose-C kg^{-1} soil had been added to each bottle. Calculated slopes were 110, 177, and 131 mg $\text{NO}_3\text{-N kg}^{-1}$ soil hr^{-1} for the 50, 150 and 250 mg $\text{KNO}_3\text{-N kg}^{-1}$ soil treatments, respectively. These slopes are 62%, 62%, and 55% higher than for the corresponding nitrate subtreatments in the 150 mg glucose-C kg^{-1}



	50 mg $\text{KNO}_3\text{-N kg}^{-1}$	150 mg $\text{KNO}_3\text{-N kg}^{-1}$	250 mg $\text{KNO}_3\text{-N kg}^{-1}$
Slope	41.75 (3.11)	61.55 (3.14)	60.45 (2.37)
Critical Point	61.62 (2.8)	100.45 (3.71)	
Intercept	175.35 (115.08)	-83.73 (183.11)	398.02 (185.34)
R^2	0.94	0.96	0.96

Fig. 4.9. Nitrous oxide production over time, associated regression lines, and model parameters for samples incubated with 50, 150, and 250 mg $\text{KNO}_3\text{-N kg}^{-1}$ soil. Glucose was added at a rate of 150 mg kg^{-1} soil. Numbers in parentheses are standard errors of the estimates.



	50 mg KNO ₃ -N kg ⁻¹	150 mg KNO ₃ -N kg ⁻¹	250 mg KNO ₃ -N kg ⁻¹
Slope	110.01 (18.09)	177.31 (24.62)	137.17 (10.18)
Critical Point	26.98 (2.17)	40.41 (3.14)	62.19 (2.89)
Intercept	-617.23 (286.14)	-974.55 (555.08)	-238.38 (376.35)
R ²	0.99	0.90	0.88

Fig. 4.10. Nitrous oxide production over time, associated regression lines, and model parameters for samples incubated with 50, 150, and 250 mg KNO₃-N kg⁻¹ soil. Glucose was added at a rate of 300 mg kg⁻¹ soil. Numbers in parentheses are standard errors of the estimates.

soil treatment. The critical point for the 50 mg $\text{KNO}_3\text{-N kg}^{-1}$ soil subtreatment occurred 67 hours sooner than the corresponding critical point in the 0-glucose-carbon treatment, and 33 hours sooner than for the 150-glucose-carbon experiment. The critical point for the 150 mg $\text{KNO}_3\text{-N kg}^{-1}$ soil subtreatment occurred 88 hours sooner than the corresponding critical point for the 0 glucose-carbon treatment and 60 hours sooner than the corresponding critical point for the 150 mg glucose-C kg^{-1} soil. A critical point that occurred after 62 hours of incubation was observed for the 250 mg $\text{KNO}_3\text{-N kg}^{-1}$ soil treatment.

Conclusions

It is obvious that quantifying the effect of temperature on denitrification may be complicated by the temperature regulation of other processes such as nitrogen mineralization, a process that consumes oxygen and thus promotes the occurrence of anaerobic microsites. The influence of temperature on oxygen solubility is also of importance, since oxygen is one of the principal regulators of denitrification in the system under study. As seen in this study, there is an interaction between temperature and substrate concentration, with control samples having lower denitrification rates than carbon-amended samples. Use of the Arrhenius equation to quantify the effect of temperature on denitrification should be done with caution, since such an approach does not take into consideration such interactions. The Arrhenius equation was derived from thermodynamic consideration of chemical reactions, where the concentration and nature of the reactants and products can be accurately known. That is not the case for microbial processes such as denitrification.

Additionally, denitrifier species common at 25 °C are probably different in terms of physiology and environmental requirements from those of the thermophilic bacteria living at 45 or 50 °C. Bremner and Shaw (1958), and Nommik (1956) reported optimum temperatures for denitrification of approximately 65 °C. Nitrous oxide evolution at such high temperatures may not necessarily be the result of denitrifiers but also of chemodenitrification, the chemical production of nitrous oxide. A more detailed study of the temperature regulation of denitrification is needed, since the current knowledge was developed based on methodology no longer employed in denitrification studies.

The use of organic amendments also increases the water holding capacity of a soil, and indirectly promotes the occurrence of anaerobic organisms by the resultant increase in soil respiration. The proximity of the water table to the soil surface may also contribute to this effect. As this study shows, the rate of denitrification will reach a maximum at saturation conditions such as those observed after a rainfall event, and it appears that moisture content is the main regulator of denitrification in the system under study.

Organic amendments also add organic carbon to the soil, which results in increased microbial growth by providing an energy source for microbes. As observed in this study, denitrifiers were able to metabolize added nitrate faster when an appropriate concentration of carbon was supplied. Actually, the rate of denitrification was more highly correlated to soluble carbon concentration than it was to nitrate. In a sandy soil this is important, since such soils are normally low in soluble forms of carbon which, together with oxygen availability, regulate denitrification.

There is increasing interest in the use of organic amendments for crop production. The benefits of such practices are well known. It was shown here that the denitrifier population in a sandy soil under study has the potential to metabolize $50 \text{ mg NO}_3^- \text{ kg}^{-1}$ soil in 27 hours, as long as the conditions are optimum for denitrification to proceed. Such conditions include low soil oxygen concentrations such as those occurring after rainfall events, nitrate coming from fertilizer applications, and soluble organic carbon including that provided by organic amendments. Low organic carbon concentrations, in addition to very short-term reducing conditions, will not likely enable microbes to compete with plant roots for nitrate to the extent of limiting plant access to this nutrient. However, additions of organic amendments may increase the potential for denitrification to occur.

CHAPTER 5

MEASURED AND SIMULATED DENITRIFICATION IN SEVERAL FLORIDA SOILS AMENDED WITH ORGANIC WASTES

Introduction

As previously stated, additions of organic amendments to soils promote microbial growth as a consequence of the addition of an energy source for microorganisms. Organic amendments to sandy soils also increase their water holding capacity, by increasing the porosity of the soil. The increase in microbial growth, together with the increase in soil porosity, could result in higher denitrification rates as compared to those for unamended soils.

The fate of applied N, whether through the use of chemical fertilizers or the land application of organic amendments, and the extent to which such applications affect the rate of denitrification in soils, is of relevance and concern to growers and scientists. Continued research in this area is specially important, due to the increasing popularity of land application of organic wastes to croplands.

Field measurements of denitrification are characterized by a high degree of spatial and temporal variability, with coefficients of variation typically above 100%. Such spatial variability is a result of normal heterogeneity in the soil's physical, chemical, and biological properties, coupled with the high sensitivity of the denitrification process to

such parameters. The temporal variability is caused by climatic factors such as rainfall and temperature, and cultural practices such as fertilization, irrigation and tillage.

Simulation models (a series of coupled mathematical equations) can serve as an effective tool to increase our visualization and understanding of the many transformations and pathways N may undergo. There is a wide variety of models developed to understand a given process. There are *empirical* models that basically describe a process in statistical terms, while *mechanistic* models, in addition, help us to understand the process. A *dynamic* model, in contrast to a *static* model, includes time as a variable. There are also *deterministic* models, with predictive capabilities of a quantitative nature. A *stochastic* model, on the other hand, not only has predictive capabilities but can also provide a probability distribution along with the predictions.

There are models that simulate the entire N cycle in soils, with each model having denitrification subroutines which generally provide at least qualitative estimates of rates of denitrification. Several simulation models have been developed to provide more quantitative estimates of denitrification rates in soils as well. Early denitrification models were developed for use at the laboratory scale (Focht, 1974; Mehran and Tanji, 1974). More recent versions (Johnsson et al., 1991; Li et al., 1992) tend to be field-scale models that provide estimates of either total denitrification or individual rates of N_2 and N_2O evolution. There are also simulation models which characterize the spatial variability of denitrification based on a landscape classification system (Elliot and Jong, 1992).

The objectives of this chapter are: (1) to obtain estimates of the maximum potential denitrification rate from soils amended with organic wastes and also for

unamended soils; (2) measure denitrification rates in intact soil cores collected periodically from amended and unamended soils; and (3) incorporate resultant data from objectives 1 and 2 into a suitable simulation model.

Materials and Methods

Sample Collection and Handling

Soil samples for denitrification measurements were collected from several regions in Florida (Fig. 5.1). Soil cores and bulk samples, for preliminary studies, were collected from a deep sand planted to citrus, and also from a plastic-mulched tomato bed near the city of Bradenton in Manatee County. Samples from the tomato beds were collected at the University of Florida Gulf Coast Research and Education Center, where tomato is normally subirrigated by maintaining a perched water table at the 45 cm depth. Similarly, soil samples were collected from a shallow water-table soil planted to citrus near the city of Boynton Beach, in Palm Beach County. This soil was amended yearly with domestic dewatered sewage sludge.

Later, intact soil cores as well as bulk samples were collected from the center of a plastic-mulched tomato bed, amended with a rate equivalent to 30 Mg ha⁻¹ municipal solid waste (MSW) compost (Bradenton site). Total elemental concentrations in the compost used for this study are presented in Table 5.1. Elements were analyzed according to USEPA method 3050 (USEPA, 1982). Concentrations for all elements of concern were below the maximum allowable concentrations for unrestricted land application, according to Florida Administrative Code Section 17-709. Compost was roto-tilled 20 cm deep,



Fig. 5.1. Location of the sites where samples were collected for denitrification measurements.

Table 5.1. Mean elemental analysis and associated standard deviations of the MSW-compost used in this study (n=6).

Element	Concentration	Element	Concentration
	mg kg ⁻¹		mg kg ⁻¹
N	8800 ± 1000	Cu	163 ± 6
P	2420 ± 71	Mn	243 ± 9
K	3710 ± 42	Al	12800 ± 283
Ca	25850 ± 1485	Na	5245 ± 64
Mg	2925 ± 290	Cd	7 ± 1
Fe	10450 ± 495	Ni	48 ± 2
Zn	595 ± 8	Pb	285 ± 23

fifteen days before planting. This material was relatively low in N, with a C:N ratio of 23.

Tomato was fertilized following IFAS recommendations, calling for 212 Kg N ha⁻¹ each season, in two surface bands along the bed. A set of four sampling wells was placed in the bed center (two in the control, and two in the amended bed), to monitor the movement of nitrate through the soil profile to a depth of 90 cm below the water-table. Water samples were collected biweekly, at 15 cm intervals, starting from the top of the water table. They were analyzed for nitrate using a rapid flow analyzer (RFA; APHA, 1989). However, they always tested below detection limits.

Intact soil cores and bulk samples were concurrently collected from a deep sand planted to citrus and amended with a rate equivalent to 55 Mg ha⁻¹ MSW compost near the city of Okeechobee (Okeechobee site). Cores were collected from the drip lines of trees from both amended and unamended sections of the grove, throughout a 12 month period.

During each sampling event, ten intact soil cores (20 cm length, 5 cm diameter) were collected from each of the treatments. Acetate sleeves housed in a polyvinylchloride (PVC) probe were driven into the soil using a 2 kg sledge hammer. The core's dimensions were selected based on results obtained by Parkin (1990). He concluded that, in order to encompass a significant portion of the spatial variability affecting denitrification, more than 5 kg of soil should be collected from each treatment. Approximately 7 kg of soil were collected during each sampling event, making sure cores showed less than 10% compaction. Soil cores used in the present study were kept in an ice chest and transported to Gainesville, where they were incubated within 24 hours of collection.

Maximum Potential Rate of Denitrification

Smith and Tiedje (1979) proposed the occurrence of two distinct denitrification phases after soil is exposed to anaerobic conditions under laboratory conditions. During phase I, if the chemical chloramphenicol is added, the production of new denitrifying enzymes is inhibited; consequently, phase I provides an estimate of the response of a given soil, including existing enzymes, to short-term anaerobic conditions. Phase II, on the other hand, relates to more long-term conditions where the subsequent production of new denitrifying enzymes is taken into consideration as well. For the present studies, phase I was the primary source of information, since denitrification in an agricultural soil is often driven by irrigation or rainfall events which induce fluxes of nitrate and relatively short-term anaerobic conditions.

Phase I was measured using the acetylene blockage technique (Yoshinari and Knowles, 1976). Briefly, twenty five grams of soil, in triplicate, are placed in serum bottles along with 25 mL of a solution containing glucose (as a carbon source), KNO_3 (a nitrate source) and chloramphenicol, which inhibits the production of new enzymes. Purified commercial-grade acetylene (Hyman and Arp, 1987) is then added to achieve a final concentration of 10% acetylene (10 KPa) in the headspace. The acetylene inhibits the reduction of N_2O to N_2 , allowing more accurate estimation (by gas chromatography) of N_2O production during denitrification.

Serum bottles were tightly capped and a syringe needle was attached to a two-way air-tight stopcock (Cole Palmer, USA) which passed through the cap. All incubations were conducted in the dark in an incubator shaking at 180 strokes per minute. Four mL of

the headspace were collected periodically through the stopcock and stored in pre-evacuated vials (Becton Dickinson, USA) for further analysis.

Intact Soil-Core Denitrification Rates

Before incubation, soil cores were taken out of the refrigerator and allowed to adjust to room temperature. Each core then was placed in a larger-diameter PVC tube, stoppered at both ends (Fig. 5.2). Acetylene was added to achieve a concentration of 10% (10 KPa) of the gas phase, with cores then being sampled at preselected time intervals. After a lag phase of 2 to 3 hours, the production of gas remains fairly linear (Tiedje et al., 1989). Cores were typically sampled 3, 6, and 9 hours after injection of acetylene, by taking 4 mL headspace samples and placing them in 3 mL pre-evacuated vials.

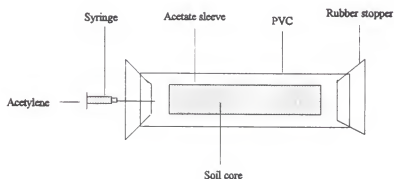


Fig. 5.2. Static soil core as used in this study, for the measurement of denitrification.

Nitrous Oxide Analysis

Gas samples were analyzed for nitrous oxide on a Shimadzu gas chromatograph equipped with a ^{63}N electron capture detector, and fitted with a packed 3.2 mm by 1.8 m stainless steel column (Porapak Q). Operating conditions included: column temperature 30 °C; ECD temperature 300 °C; injector temperature 110 °C; 5% CH_4 / 95% argon carrier gas. The amount of N_2O dissolved in the soil water was calculated using the Bunsen absorption coefficient, according to the relationship (Tiedje, 1982)

$$M = C_g (V_g + V_l \alpha),$$

Here M = amount of N_2O in the water plus gas phase, C_g = concentration of N_2O in the gas phase, V_g = volume of the gas phase, V_l = volume of the liquid phase, and α = the Bunsen absorption coefficient.

Data were tested for normality and, when necessary, the variance was stabilized using a \log_{10} transformation. For transformed data, "actual rates" were calculated by multiplying the detransformed values by a correction factor (Cornell, 1991). This factor is equal to *antilog* ($\text{MSE} / 2$), where MSE is the residual mean square obtained from the respective ANOVA table.

Simulation of Denitrification

The denitrification subroutine of the LEACHM model (Hutson and Wagenet, 1992) was used to simulate denitrification rates for surface soil at the sites under study. The denitrification subroutine in such models is based on the work of Johnsson and coworkers (Johnsson et al., 1991). This model assumes that the soluble-carbon concentration in soil is not limiting the process, and that denitrification is regulated by soil

aeration, the potential rate of denitrification, temperature and nitrate concentration such that:

$$\text{Denitrification} = DEA * TEF * WEF * NEF$$

Here *DEA* is the maximum denitrification potential ($\text{ng N}_2\text{O-N g}^{-1} \text{ hr}^{-1}$); *TEF* is the effect of temperature; *WEF* is the soil water effect, and *NEF* is the effect of soil nitrate concentration.

The effect of temperature can be described as:

$$TEF = Q_{10} \left[\frac{T_s - T_b}{10} \right]$$

where T_s is the soil temperature ($^{\circ}\text{C}$) and T_b is the temperature ($^{\circ}\text{C}$) at which $TEF = 1$.

The soil water effect relationship used here was developed based on results reported in Chapter 4. The water relationship for the control bed was:

$$WEF = \frac{0.09}{0.09 + 0.91 + [2.718 + \exp(-0.50 * (\theta - 23))]}$$

while the relationship for the amended bed was:

$$WEF = \frac{0.05}{0.05 + 0.95 + [2.718 + \exp(-0.36 * (\theta - 20))]}$$

Here, θ is the volumetric soil water content (%).

The effect of nitrate concentration is controlled by the half saturation constant C_s for Michaelis-Menten kinetics, such that:

$$NEF = \left[\frac{N_s}{N_s + C_s} \right]$$

Here N_s is the concentration of nitrate in the soil (mg L^{-1}), and C_s is the concentration at 50% of the maximum rate under optimum conditions.

Results and Discussion

Soil samples were collected from several geographical locations, each typifying different cropping systems. Tomato is grown on raised plastic-mulched beds which are irrigated by maintaining a perched water table, typically between 45 and 75 cm (Fig. 5.3). The proximity of the water table to the soil surface at the tomato site increases the chances for denitrification to occur at a rate higher than at the citrus site.

The citrus site consisted of a mature orange grove planted on a deep sand with the water table between 1 and 1.5 m deep. Irrigation, at this site, is accomplished through microsprinklers located near the trunk of each tree.

Preliminary Studies

Soil bulk samples were collected at the Palm Beach site and incubated for denitrifying enzyme activity (DEA) measurements. Results suggested that the majority of denitrifying activity was located in the top 15 cm of soil (Table 5.2). The DEA rate for the top 15 cm of soil, four months after the final sludge application, was significantly higher ($P = 0.05$) than the rate for the same depth increment in the control plots. The DEA rates calculated for the sludge-amended plots at the 15-30 and 30-45 cm depths were not statistically different from corresponding rates in the control plots. The effect of the organic amendment on the growth of denitrifiers was apparent. The addition of a carbon source seems to have prompted a considerable long-term increase in the activity of

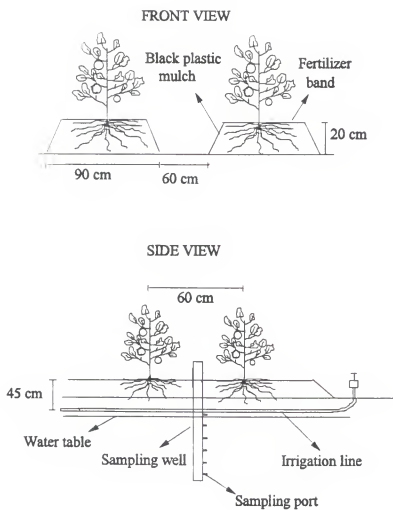


Fig. 5.3. Tomato production systems at the Bradenton site. Water samples were collected through the sampling wells for nitrate analysis.

Table 5.2. Maximum denitrification potential rates (DEA) at the Palm Beach site, which was amended with sewage sludge at an equivalent rate of 7 to 8 Mg ha⁻¹ yr⁻¹ during three years (n = 9).

Depth (cm)	Linear Equation	R ²
----- ng N ₂ O-N g ⁻¹ hr ⁻¹ -----		
Amended		
0 - 15	ng N ₂ O-N = -33.2 + 54.3 T	0.82
15 - 30	ng N ₂ O-N = 1.44 + 3.63 T	0.68
30 - 45	ng N ₂ O-N = 4.35 + 1.03 T	0.61
Control		
0 - 15	ng N ₂ O-N = -6.5 + 12.02 T	0.98
15 - 30	ng N ₂ O-N = 3.93 + 1.55 T	0.78
30 - 45	ng N ₂ O-N = 3.85 + 1.55 T	0.82

denitrifiers.

Intact soil cores were also collected from the amended plots and incubated as previously described. When the soil-core rates were plotted against volumetric moisture content (Fig. 5.4), a strong correlation between denitrification and moisture content was apparent. Interestingly, many of the cores with high volumetric moisture content (16 - 25%) at this site produced relatively higher amounts of N₂O gas than cores collected from the same area, whether they had received sludge previously or not. This observation was particularly important for this site, since the water table was maintained near the soil surface, between 45 - 60 cm. Rates were also calculated for samples collected from a deep sand planted to citrus in Manatee County, with a water table several meters deep. Intact

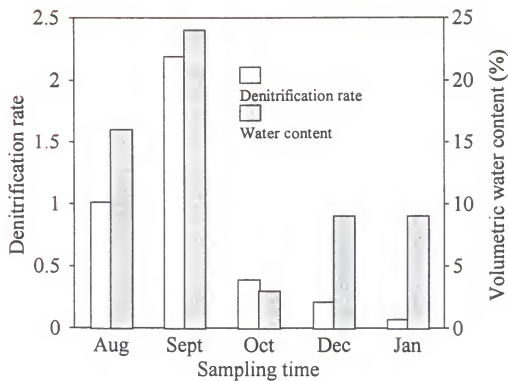


Fig. 5.4. Relationship between soil moisture content and denitrification rate for soil cores collected from the Palm Beach site. Denitrification rates are given as ng N₂O-N g⁻¹ h⁻¹.

soil cores were collected from the drip lines of trees as well as from the row middles, which were covered with grass.

Denitrification rates at this site followed the same pattern as those at the Palm Beach site, with the highest rates observed during the September sampling, and then decreasing as the year progressed (Table 5.3). There was a tendency for the rates obtained from the grassed row middles to be higher than those obtained from the drip line. This difference could be due to a higher water content, and also to higher carbon availability in the grassed rows.

Denitrification Measurements at Sites Amended with MSW-Compost

Measurements at the Bradenton site. Denitrification rates were measured periodically, at Bradenton, during the two tomato growing seasons of 1995. The tomato growing season in Bradenton normally starts near the end of February or the beginning of March, and ends in the middle of June. It starts again near the end of August or the beginning of September, and ends during the last two weeks of December.

Denitrification rates for intact soil cores collected from a tomato bed amended with MSW-compost and a bed used as a control are presented in Table 5.4, with the majority of the data requiring a log transformation to stabilize the variance.

Measured rates for both the control and amended beds can be separated into three distinct groups. The first group includes sampling days 87 to 115 (March and April), where an average denitrification rate of $0.57 \pm 0.05 \text{ ng N}_2\text{O-N g}^{-1} \text{ hr}^{-1}$ was calculated for the control bed. An average rate of $0.26 \pm 0.17 \text{ ng N}_2\text{O-N g}^{-1} \text{ hr}^{-1}$ was measured for the amended bed during the same period of time. The second group includes sampling days

Table 5.3. Intact soil-core rates for the citrus site in Bradenton ($n = 36$). This site did not receive any organic amendments. Rates are given in $\text{ng N}_2\text{O-N g}^{-1} \text{ hr}^{-1}$.

Date	Linear Equation	$R^{2\dagger}$	Actual Rate
----- Drip Line -----			
September	$\text{ng N}_2\text{O-N} = 2.7 + 0.93 \text{ T}$	0.59	0.93
October	$\log \text{ng N}_2\text{O-N} = 0.07 + 0.066 \text{ T}$		0.40
December	$\log \text{ng N}_2\text{O-N} = -0.46 + 0.023 \text{ T}$		0.02
January	$\text{ng N}_2\text{O-N} = 0.36 + 0.023 \text{ T}$	0.52	0.03
----- Middle Row -----			
September	$\text{ng N}_2\text{O-N} = 3.51 + 1.09 \text{ T}$	0.58	1.09
October	$\log \text{ng N}_2\text{O-N} = 0.64 + 0.05 \text{ T}$		0.54
December	$\log \text{ng N}_2\text{O-N} = -0.44 + 0.02 \text{ T}$		0.08

[†] R-squares for the log-transformed data are not shown, since they will tend to be artificially higher.

142 to 234 (late May to late August). Here, average rates of 0.8 ± 0.51 and 2.84 ± 1.65 $\text{ng N}_2\text{O-N g}^{-1} \text{ hr}^{-1}$ were measured for the control and amended beds, respectively. The third group includes days 257 to 342 (mid September to mid December). For this last group, average rates of 0.6 ± 0.36 and 0.98 ± 0.15 $\text{ng N}_2\text{O-N g}^{-1} \text{ hr}^{-1}$ were calculated for the control and amended beds, respectively. This grouping correlates well with an average volumetric water content of 21 and 22% for the control and amended beds of the first group, respectively; 25 and 24 % for the second group; and 21 and 22 % for the third group.

Maximum potential rates (DEA) fluctuated between 1 and 47 $\text{ng N}_2\text{O-N g}^{-1} \text{ h}^{-1}$ for the control bed, and between 8 and 95 $\text{ng N}_2\text{O-N g}^{-1} \text{ hr}^{-1}$ for the amended bed (Table 5.5).

Table 5.4. Denitrification rates for intact soil cores collected during 1995, for a tomato bed amended at a rate equivalent to 30 Mg ha⁻¹ MSW compost and a bed with no organic amendments. (n = 30).

Julian Date	Linear Equation	R ^{2†}	Actual Rate
----- ng N ₂ O-N g ⁻¹ hr ⁻¹ -----			
Amended			
87	log ng N ₂ O-N = -0.15 + 0.079 T		0.41
102	log ng N ₂ O-N = -0.34 + 0.037 T		0.07
115	log ng N ₂ O-N = -0.31 + 0.08 T		0.29
142	ng N ₂ O-N = 0.065 + 4.06 T	0.55	4.06
157	ng N ₂ O-N = -4.18 + 4.94 T	0.75	4.94
173	log ng N ₂ O-N = -0.04 + 0.097 T		0.89
200	ng N ₂ O-N = -4.12 + 2.51 T	0.63	2.51
234	ng N ₂ O-N = -4.4 + 1.79 T	0.64	1.79
257	log ng N ₂ O-N = -0.32 + 0.12 T		0.85
289	ng N ₂ O-N = 0.23 + 0.99 T	0.54	0.99
325	log ng N ₂ O-N = 0.59 + 0.057 T		1.19
342	log ng N ₂ O-N = 0.37 + 0.063 T		0.88
Control			
87	log ng N ₂ O-N = 0.014 + 0.073 T		0.52
102	log ng N ₂ O-N = 0.22 + 0.061 T		0.58
115	log ng N ₂ O-N = -0.32 + 0.11 T		0.62
142	log ng N ₂ O-N = 0.26 + 0.089 T		1.37
157	log ng N ₂ O-N = 0.81 + 0.5 T		0.74
173	log ng N ₂ O-N = -0.46 + 0.026 T		0.03
200	ng N ₂ O-N = -1.1 + 0.72 T	0.71	0.72
234	log ng N ₂ O-N = -.28 + 0.13 T		1.14
257	log ng N ₂ O-N = -0.44 + 0.12 T		0.57
289	ng N ₂ O-N = 1.08 + 0.14 T	0.51	0.14
325	log ng N ₂ O-N = 0.84 + 0.037 T		1.03
342	log ng N ₂ O-N = 0.29 + 0.06 T		0.64

†R-squares for the log-transformed data are not shown, since they will tend to be artificially higher.

DEA rates for the amended bed were significantly higher ($P = 0.05$) than for the control bed, with the exception of the first sampling event. It was evident that there was a lesser degree of variability in the data as compared with rates measured from the intact soil cores. This difference is due mostly to the fact that DEA measurements were obtained from soil slurries, while the cores were incubated under similar soil moisture conditions to those found in the field. Potential rates of denitrification for both the control and amended beds were between 8 and 100 times higher than corresponding rates for the soil cores. These rates suggest that the potential of these soils to denitrify is high, and that higher soil-core rates could be expected under near-optimum conditions.

The denitrification process is regulated by several environmental factors, with the individual effects of each regulator being as important as the interaction among them. In that sense, it may be difficult to explain the reason for the observed differences in the measured denitrification rates. However, there are trends in the behavior of some of the regulators that can help to explain the differences in observed denitrification rates.

Denitrification was probably not controlled by water-soluble organic carbon (OC), since water-soluble OC concentrations remained relatively constant throughout the sampling period (Fig. 5.5). However, denitrification rate in the amended bed was likely affected by the fact that the compost had been applied soon after it was received from the composting plant. Compost thus might have been at or near its peak in the production of organic acids when applied. Plants in the amended bed were uniformly stunted following the application. High levels of organic acids are known to cause phytotoxic response, specially in seedlings (Zucconi et al., 1981). Plants in the amended bed never did fully

Table 5.5. Denitrifying enzyme activity (DEA) measurements obtained during 1995 for the top 20 cm of a tomato bed amended with compost, and also for a bed with no amendments (n =9).

Julian Date	Linear Equation	R ²
----- ng N ₂ O-N g ⁻¹ h ⁻¹ -----		
Amended		
102	ng N ₂ O-N = 4.79 + 8.38T	0.81
115	ng N ₂ O-N = 3.07 + 30.13T	0.97
142	ng N ₂ O-N = 10.28 + 95.61T	0.88
173	ng N ₂ O-N = -1.08 + 11.75T	0.89
234	ng N ₂ O-N = 3.2 + 45.26T	0.90
289	ng N ₂ O-N = 2.65 + 48.34T	0.92
325	ng N ₂ O-N = 5.64 + 53.54T	0.90
Control		
102	ng N ₂ O-N = 3.28 + 10.85T	0.99
115	ng N ₂ O-N = 3.38 + 4.97T	0.95
142	ng N ₂ O-N = 6.31 + 47.28T	0.91
173	ng N ₂ O-N = 1.86 + 1.0T	0.82
234	ng N ₂ O-N = 2.84 + 12.5T	0.88
289	ng N ₂ O-N = 1.54 + 15.18T	0.87
325	ng N ₂ O-N = 3.33 + 10.54T	0.90

recover, though neither did they die. It has been reported that acetic acid inhibits denitrification for short periods of time (Lescure et al., 1992; Schipper, 1991).

Nitrate concentration in soil apparently did not limit denitrification to a sizeable extent, since NO₃-N concentrations in soil were relatively low when the highest

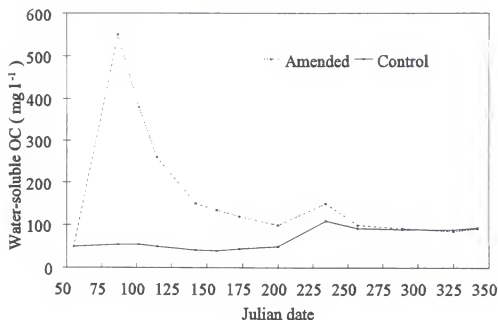


Fig. 5.5. Water-soluble organic carbon (OC) concentrations in the tomato beds at the Bradenton site during this study.

denitrification rates for the soil cores were observed. In fact, nitrate is not considered a limiting factor for denitrification in agricultural soils (Parkin and Robinson, 1989).

However, preliminary studies showed that measured rates were 2.5 times higher for samples collected from the fertilizer band than those measured at the center of the bed.

Denitrification follows a first-order rate with respect to nitrate, specially under high electron-donor pressure (Cho, 1982; Reddy et al., 1980). Overall, however, denitrification tends to follow a second-order rate. The process is also regulated by water soluble OC, temperature and oxygen concentration.

The highest rates were measured during the summer months, which also includes the months with the heaviest precipitation of the year. As a consequence, it appears that soil moisture content (oxygen concentration) is the main regulator of the process in tomato beds. This hypothesis agrees with several reported studies for agricultural soils (Aulakh et al., 1984; Mosier et al., 1986; Svensson et al., 1991).

Measurements at the Okeechobee site. Intact soil core denitrification rates for the Okeechobee site (Table 5.6) were consistently lower than those measured for tomato beds at the Bradenton site, but were comparable to those measured for the deep-sand citrus site near Bradenton. Soil-core rates for the control plot oscillated between a low of $0.04 \text{ ng N}_2\text{O-N g}^{-1} \text{ hr}^{-1}$ measured during June of 1995 and January of 1996 and a high of $0.22 \text{ ng N}_2\text{O-N g}^{-1} \text{ hr}^{-1}$ measured for May of 1996. Rates for the amended plot fluctuated between a low of $0.07 \text{ ng N}_2\text{O-N g}^{-1} \text{ hr}^{-1}$ measured during June and July of 1995, and a high of $0.44 \text{ ng N}_2\text{O-N g}^{-1} \text{ hr}^{-1}$ measured during March of 1996.

Maximum potential denitrification rates (DEA) for both the control and amended plots are presented in Table 5.7. DEA rates for the amended plot were significantly higher ($P = 0.05$) than respective DEA rates for the control for all cases except the October sampling. There was a trend for the lowest rates to occur during the months of December and January, while the highest rates were measured during the sampling events in March and May for both the control and amended plots.

Maximum potential rates measured at Okeechobee were between 11- and 27-fold higher than intact soil-core rates for the control plots during the same sampling events,

Table 5.6. Denitrification rates for soil cores collected during 1995, for the top 20 cm of a soil planted to citrus near Okeechobee. A section of the grove was amended with a rate equivalent to 50 Mg ha⁻¹ MSW compost. (n = 30).

Sampling Date	Linear Equation	R ²	Actual Rate
----- ng N ₂ O-N g ⁻¹ hr ⁻¹ -----			
Amended			
June	$\log \text{ ng N}_2\text{O-N} = -0.35 + 0.037 \text{ T}$		0.07
July	$\log \text{ ng N}_2\text{O-N} = -0.23 + 0.033 \text{ T}$		0.07
August	$\log \text{ ng N}_2\text{O-N} = -0.2 + 0.044 \text{ T}$		0.11
September	$\log \text{ ng N}_2\text{O-N} = -0.34 + 0.13 \text{ T}$		0.27
October	$\log \text{ ng N}_2\text{O-N} = -0.33 + 0.06 \text{ T}$		0.13
December	$\text{ng N}_2\text{O-N} = 3.19 + 0.16 \text{ T}$	0.82	0.16
January	$\log \text{ ng N}_2\text{O-N} = -0.35 + 0.050 \text{ T}$		0.09
March	$\log \text{ ng N}_2\text{O-N} = -0.19 + 0.17 \text{ T}$		0.65
May	$\log \text{ ng N}_2\text{O-N} = -0.42 + 0.066 \text{ T}$		0.25
Control			
June	$\log \text{ ng N}_2\text{O-N} = -0.28 + 0.025 \text{ T}$		0.04
July	$\log \text{ ng N}_2\text{O-N} = -0.25 + 0.033 \text{ T}$		0.07
August	$\log \text{ ng N}_2\text{O-N} = -0.2 + 0.038 \text{ T}$		0.09
September	$\log \text{ ng N}_2\text{O-N} = -0.34 + 0.04 \text{ T}$		0.11
October	$\log \text{ ng N}_2\text{O-N} = -0.37 + 0.06 \text{ T}$		0.18
December	$\log \text{ ng N}_2\text{O-N} = 2.64 + 0.11 \text{ T}$		0.11
January	$\text{ng N}_2\text{O-N} = -0.33 + 0.041 \text{ T}$	0.75	0.04
March	$\log \text{ ng N}_2\text{O-N} = -0.28 + 0.06 \text{ T}$		0.15
May	$\log \text{ ng N}_2\text{O-N} = -0.35 + 0.07 \text{ T}$		0.22

Table 5.7. Maximum potential denitrification rates (DEA) for soil collected during 1995, for the top 20 cm of a soil planted to citrus near Okeechobee. A section of the grove was amended with rate equivalent to 50 Mg ha⁻¹ MSW compost (n = 9).

Sampling Date	Linear Equation	R ²
----- ng N ₂ O-N g ⁻¹ hr ⁻¹ -----		
Amended		
June	ng N ₂ O-N = 2.4 + 4.2 T	0.92
July	ng N ₂ O-N = -0.76 + 4.7 T	0.90
August	ng N ₂ O-N = 0.66 + 6.6 T	0.86
September	ng N ₂ O-N = 1.12 + 5.4 T	0.93
October	ng N ₂ O-N = 0.33 + 3.6 T	0.90
December	ng N ₂ O-N = -0.3 + 4.1 T	0.88
January	ng N ₂ O-N = 0.8 + 2.6 T	0.85
March	ng N ₂ O-N = 0.35 + 8.17 T	0.91
May	ng N ₂ O-N = 0.74 + 7.14 T	0.87
Control		
June	ng N ₂ O-N = 3.9 + 1.1 T	0.81
July	ng N ₂ O-N = 2.7 + 1.8 T	0.89
August	ng N ₂ O-N = 2.4 + 2.3 T	0.91
September	ng N ₂ O-N = 3.0 + 1.6 T	0.84
October	ng N ₂ O-N = 0.37 + 2.4 T	0.91
December	ng N ₂ O-N = 0.95 + 1.2 T	0.87
January	ng N ₂ O-N = 0.25 + 1.1 T	0.89
March	ng N ₂ O-N = 1.20 + 4.3 T	0.86
May	ng N ₂ O-N = 2.2 + 4.2 T	0.84

and between 13- and 29-fold higher for the amended plots during the months of September 1995 to May 1996. However, for the months of June, July, and August 1995 (1, 2, and 3 months after sludge application respectively), DEA rates for the amended plots were between 60- and 67-fold higher than corresponding soil-core rates. This difference is probably the result of an organic-amendment effect on microbial growth.

There was no apparent difference between soil-core rates calculated for the amended and control plots. However, DEA rates for the amended plot were significantly higher (2- to 4-fold higher) than DEA rates calculated for the control plot for the same sampling dates.

Soil moisture content at the time of sampling was relatively low during most of the sampling events. This situation probably contributed to the low rates observed for the soil cores.

Simulation of Denitrification

The model DNDC5.8 (Denitrification-Decomposition; Li et al., 1992) was originally chosen to simulate denitrification rates under Florida soil and climatic conditions. One of the reasons for choosing this model was that it had been previously validated, with few modifications to the original model and using soil and weather data from the Everglades Agricultural Area (EAA) in south Florida. Although the model appeared to simulate adequately the rate of denitrification for organic soils of the EAA (Li et al., 1994), that was not the case for the data obtained in this study. Denitrification models tend to be site-specific, since the process is highly sensitive to soil temperature and moisture regimes (Elliot and Jong, 1992; Parkin and Robinson, 1989). The water balance

of the DNDC model may need to be calibrated as well, to include the effect of a shallow water table such as the one maintained for the subirrigated tomato beds.

The denitrification subroutine of the LEACHN model simulates denitrification based on potential rate of denitrification, soil nitrate concentration, temperature, and soil moisture content. It assumes that easily decomposable organic carbon is available under non-limiting conditions, which is not an unrealistic assumption for a tomato crop. Only the effect of DEA, nitrate and soil aeration status are included in this study, because those are the factors assumed to be most limiting in this case. Although temperature also influences the rate of denitrification it is not included here, because all the incubations were performed under lab conditions.

A comparison of denitrification rates measured for intact soil cores with those simulated using the model LEACHN for the Bradenton site is presented in Fig. 5.6. The top graph represents measured and simulated rates for the control bed. In this case, the model tends to underestimate measured rates for all cases with the exception of measured rates for the day 173 and day 257 sampling dates. The bottom graph shows simulated and measured rates for the amended tomato bed. Contrary to the control bed, the model tended to overestimate measured values for the amended bed. However, it is important to note that the model was able to capture the temporal variability observed in the measured rates for both the control and amended beds.

The same approach was followed to simulate measured rates in the deep sand at the Okeechobee site (Fig 5.7). It is obvious that the LEACHN model did not provide reasonable estimates for either of the treatments, with the exception of the March and May

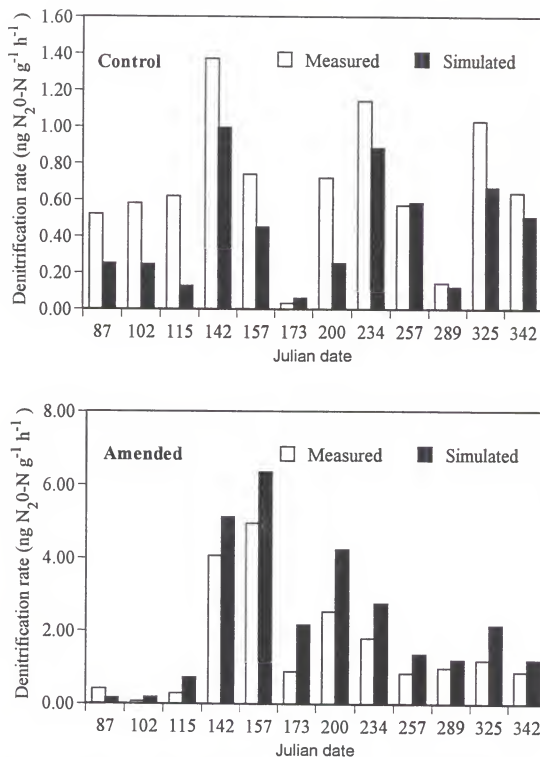


Fig. 5.6. Simulated and measured denitrification rates for samples collected from a tomato bed amended with MSW-compost and a nearby bed used as a control. The denitrification subroutine of the LEACHN model was used to obtain simulated values.

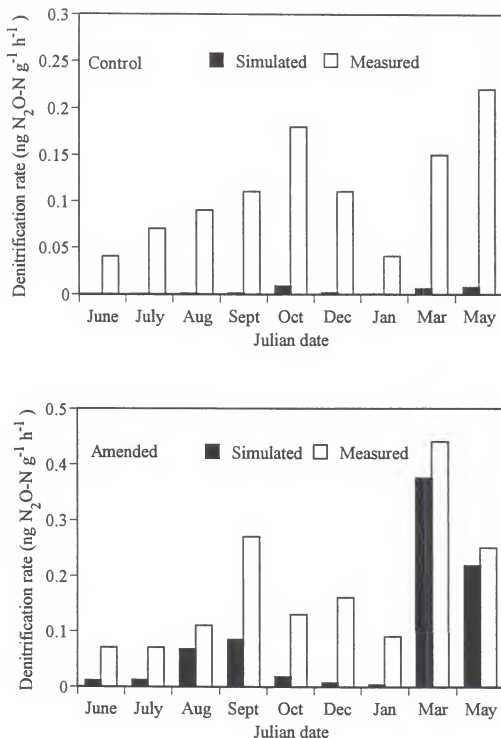


Fig. 5.7. Simulated (using LEACHN) and measured denitrification rates for a deep sand planted to citrus (Okeechobee site). Samples were collected from a section of the grove that was amended with MSW-compost and from a second section used as a control.

sampling dates for the amended plot. Samples collected during these months showed the highest moisture contents of all the sampling events combined for that location. During these sampling dates, the volumetric moisture content oscillated between 21 and 22 % while, during the rest of the sampling period, it varied between 12 and 18 %.

Regardless of the simplicity of the model used to simulate denitrification rates, it appears possible to simulate the temporal variability of the process with reasonable accuracy for subirrigated tomato beds. In the case of the Okeechobee site, however, the model did not provide accurate simulations. This may be a result of different soil moisture and nitrate relationships. Also, the assumption of non-limiting conditions for water-soluble OC may not be appropriate for a sandy soil with a water table that is 1 to 1.5 m deep. The concentration of soluble organic carbon at the tree dripline, where cores were taken, for the Okeechobee site was consistently lower (50 to 100%) than water-soluble OC concentrations at the bed center for the tomato site at Bradenton (Fig. 5.8). The distribution of soluble carbon at the Okeechobee site agrees with the typical root distribution of a citrus tree, with the majority of the roots localized in the surface soil layer.

Conclusions

It was shown here that additions of organic amendments to sandy soils, in the form of municipal solid waste compost or sewage sludge biosolids, increased the potential for denitrification. Soil-core denitrification rates were normally higher for the amended plots than for the control plots. This difference was more evident for samples collected from the tomato beds. While there was no clear trend for soil-core denitrification rates measured at

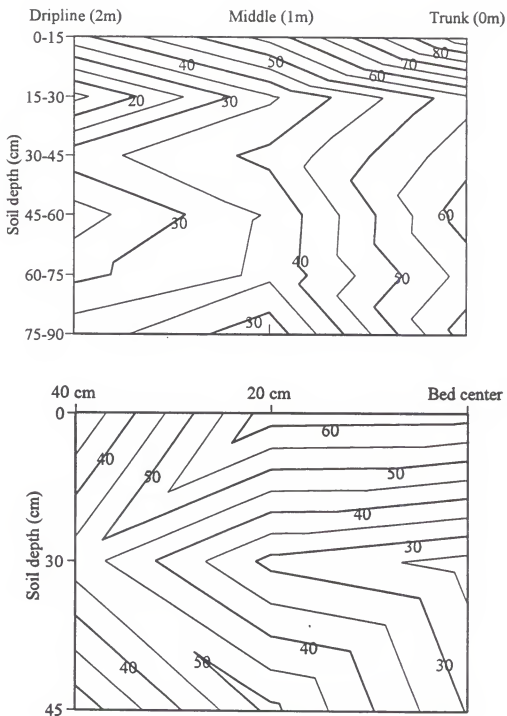


Fig. 5.8. Soluble organic carbon concentrations for the Okeechobee site (top graph), and for the tomato site at Bradenton (bottom graph) during May 1995. Contour lines represent mg water-soluble OC kg⁻¹ soil. Graphs were generated using DeltaGraph_® pro 3.5.

the Okeechobee site, DEA measurements for the amended plots were higher than those for the control plots.

Waste application rates used in these studies are relatively low; thus, the effect of organic amendment applications on the rate of denitrification would probably increase if considerably larger rates were applied. The increased use of organic amendments, in addition to proximity of the water table, created more nearly optimal conditions for denitrification to proceed at a faster rate at the tomato site in Bradenton than at the Okeechobee site.

In the study of denitrification, sampling protocols may not provide representative estimates of the dynamics for this process. Soil cores collected at the Okeechobee site were obtained during relatively dry days, which may explain the low associated gas production. Citrus trees at this location are fertigated through microsprinklers; such practice could result in considerable gas evolution during irrigation events. Thus, denitrification measurements immediately before and after an irrigation event would provide critical information to further characterize the process of denitrification for this soil.

The simulation model LEACHN provided a good estimate of measured denitrification rates for the tomato site at Bradenton, but more work is needed to incorporate this routine into more complete models, and to simulate denitrification under a range of Florida's soil and weather conditions. Deterministic models such as the one used here might not account for the high degree of variability observed in a highly variable process such as denitrification. In fact, " stochastic models may prove more informative...

" (Parkin and Robinson, 1989), since the variability in moisture distribution, which is a major cause for the observed variability in denitrification, can be better described as a frequency distribution of soil moisture measurements. Stochastic models, however, require a considerably larger number of replicate samples.

CHAPTER 6 SUMMARY AND CONCLUSIONS

Introduction

More than five million metric tons of sewage sludge and 270 million metric tons of municipal solid waste (MSW) are generated in the United States each year. Traditional methods for waste disposal, such as incineration, ocean dumping, and landfilling, are becoming increasingly more costly, and some of them are now banned. This situation is of particular concern for large communities, and for states with a high growth rate such as Florida.

Organic wastes are a source of heavy metals, but also of many of the nutrients required for plant growth. There is no method for disposal of organic wastes that guarantees 100% safety. However, when wastes are applied to croplands according to federal and state guidelines, they should not present a significant health risk to humans, crops or the environment as a whole.

In the mid to late 1970s considerable research was initiated to study crop and soil responses to organic waste applications. This increase in research was largely a result of the 1977 amendment to the 1972 Federal Water Pollution Control Act, which resulted in the Clean Water Act of 1977.

Additions of organic amendments to soils not only represent a feasible alternative for disposal, but can also be considered a good cultural practice. This practice may result in effects that are either beneficial or detrimental for plant growth. Net outcome depends on factors such as the quality of the amendment, application rate, and specific soil properties including pH, organic matter content, cation exchange capacity, water content, and soil texture.

Organic-waste applications to croplands promote initial microbial growth, due to the addition of a fresh carbon source for these organisms. Since several of the N transformations in soil are microbially-mediated, additions of wastes will affect such transformations. This is particularly true for mineralization, immobilization and denitrification.

Denitrification is the biological conversion, under depleted oxygen conditions, of nitrogen oxides including nitrate and nitrite, to the gaseous products N_2 and N_2O . This process is regulated by the oxygen, nitrate, and soluble organic concentration of each soil. Other regulators include temperature, soil pH, and salinity.

In Florida, a large portion of the state's cropland occurs on sandy soils of inherently low fertility and yet differing hydrologic regimes. Soils of the central ridge are prone to the leaching of excess fertilizer, and especially the nitrate form. Denitrification is not generally a major factor in the N balance for these soils, since most of the profile remains aerobic throughout the year. However, additions of organic amendments to the surface layer may increase the potential for denitrification, specially after irrigation or rainfall events. In contrast, many of the vegetables grown along the west coast of Florida

are irrigated by maintaining a perched water table 45 cm to 60 cm deep. Additions of organic amendments, plus the proximity of the water table, make these soils ideal biological reactors for the denitrification process. Estimates of denitrification rates under the above conditions remain scarce or nonexistent, however.

Summary

The objectives of this dissertation were:

1. To generate data showing that domestic dewatered sewage sludge can be applied at a rate of 7 to 8 Mg ha⁻¹ year⁻¹ to a mature citrus grove without observing a significant accumulation of heavy metals in the underlying soil or plant parts several years later;
2. To study the relationship between denitrification and related soil parameters for sandy soils; and
3. To study the effect of organic amendments on the rate of denitrification in sandy soils under different cultural practices.

A summary of the major conclusions as they relate to the above objectives includes:

Objective 1. The biosolids used for this study were obtained from the Boca Raton, FL wastewater treatment plant. This material consistently tested below the maximum allowable elemental concentration for unrestricted use, and is considered a "clean" sludge.

This study was conducted in a mature citrus grove in Palm Beach County, FL. There was considerable variability in the age and health condition of the grove, a situation

that likely influenced the observed results. Results of the study (Chapter 3) showed a significant difference in the concentrations of metals and nutrients existed between the 0-15 and the 15-30 cm soil depths, with the higher concentration being in the top layer.

Leaf-tissue concentrations of most nutrients and reported trace metals fell within the normal concentration ranges for citrus leaves. Only the concentrations of Ca and Mg tested significantly higher for the amended plots than for the control plots.

Nutrient and metal concentrations in juice samples collected from the amended and control plots were not different. There was considerable variation among sampling dates, however.

Based on these results, it appears that biosolids applications at a rate of 7-8 Mg ha⁻¹ to a mature citrus grove did not increase the concentration of heavy metals, including specially those of greatest concern such as Cd and Pb.

Objective 2 involved study of the regulation of denitrification for a sandy soil. In Chapter 4 of this dissertation, a series of lab experiments were set up to study the effect of variable concentrations of electron donors and acceptors on the rate of denitrification in a sandy soil. The effects of variable soil moisture content, and of temperature, on the rate of denitrification for MSW compost-amended and unamended samples were also studied.

The production of N₂O gas increased with increasing supply of electron donors (soluble carbon compounds) and electron acceptors (nitrate). Results from this part of the study showed denitrification rates increasing with increasing carbon supply under equal and variable nitrate concentrations. Such results confirmed the fact that denitrification

follows a first-order rate equation with respect to nitrate concentration. Overall, however, the process followed a second-order rate equation, since it also depends on the soluble organic carbon concentration in the soil.

The production of N_2O gas increased with increasing soil water content for both amended and control samples. Denitrification rates were negligible below 60% water-filled pore space for the control plots, and below 40% water-filled pore space for the amended samples. Rates were significantly higher for the amended samples than for the control ones. Evidently, additions of organic amendments increase the potential for denitrification by promoting microbial growth and by increasing soil water-holding capacity.

The effect of temperature on the rate of denitrification was also studied using soil slurries. There appears to be an interaction between temperature and substrate concentration, since denitrification rates measured for carbon-amended samples were higher than for unamended samples incubated at similar temperatures. The temperature dependence of a reaction has traditionally been characterized using the Arrhenius equation and a corresponding Q_{10} value (the factor by which a reaction's rate increases when temperature is raised by 10 °C). Q_{10} values of 3.0 and 2.2 were calculated for denitrification in the control and amended samples, respectively.

Objective 3 included study of the effect of organic amendments on the rate of denitrification for intact soil cores collected in sandy soils under varying cultural practices (Chapter 5). Studies were conducted at three geographical locations: in Manatee, Palm Beach, and Okeechobee counties. Organic amendments were applied at each location,

with measured potential rates of denitrification for the amended plots always being higher than corresponding rates for the control plots at each location.

Preliminary studies conducted in Palm Beach County showed a higher potential for denitrification in plots amended with sewage sludge biosolids when compared to unamended plots. There was also a strong correlation between soil-core rates and soil moisture content, with the highest rates being observed at the highest moisture contents.

Intact soil-core denitrification rates measured for a plastic-mulched tomato bed amended with MSW-compost were significantly higher than rates measured for a nearby unamended bed. Maximum potential rates of denitrification were between 8 and 100 times higher than corresponding rates for intact soil cores. Such rates suggest that the potential for these soils to denitrify is high, and that higher soil-core rates could be expected under near-optimum conditions. Intact soil-core rates at this site oscillated between an average of 0.8 and 2.84 $\text{ng N}_2\text{O-N g}^{-1} \text{ h}^{-1}$ (1.9 and 7 $\text{g N ha}^{-1} \text{ hr}^{-1}$) for the control and the amended bed respectively during the rainy season (May to August). Denitrification rates of 0.59 and 0.67 $\text{ng N}_2\text{O-N g}^{-1} \text{ h}^{-1}$ (1.4 and 1.6 $\text{g N ha}^{-1} \text{ hr}^{-1}$) were measured for the remainder of the year.

Denitrification rates measured at Okeechobee were considerably lower than those measured at the tomato site in Bradenton. Soil-core rates for the control plot oscillated between 0.04 and 0.22 $\text{ng N}_2\text{O-N g}^{-1} \text{ hr}^{-1}$ (0.1 and 0.53 $\text{g N ha}^{-1} \text{ hr}^{-1}$), and between 0.07 and 0.44 $\text{ng N}_2\text{O-N g}^{-1} \text{ hr}^{-1}$ (0.17 and 1.1 $\text{g N ha}^{-1} \text{ hr}^{-1}$) for the amended plots. Soil moisture content at the time of sampling was relatively low during most of these sampling events. This situation probably contributed to the low rates observed for the soil cores.

The denitrification subroutine of the LEACHN model was used to simulate soil-core denitrification rates. The model provided good estimates of measured rates for the tomato site at Bradenton, but not for the citrus site near Okeechobee. Different soil moisture relationships at this site compared to the tomato site were the likely cause for the lack of correspondence between measured and simulated values.

Research Needs

Although regulations for the application of sewage sludge to croplands are more stringent now than they were several years ago, and the concentrations of the elements of concern in such material have considerably decreased, the fate of sludge-borne nutrients, metals and other toxic compounds remains in need of continuing research. Questions related to metal-uptake kinetics, loading rates, sorption-desorption of metals by soil organic fractions, mineralization, and the fate of organic N and P under Florida's climatic conditions are in need of further detailed study.

It was observed in these studies that an application rate of 30 Mg MSW-compost ha⁻¹ increased the potential rate of denitrification for sandy Florida soils. However, a rate of this magnitude is only 30 to 40 % of the rate recommended by some composting facilities. Studying the effect of higher loading rates on denitrification also remains important.

A more detailed study of the effect of temperature on the rate of denitrification also remains of interest. This is particularly true for plastic-mulched production systems, where soil temperatures fluctuate during the day and can at times reach 70 °C (commonly

exceeding 50 °C). In pure culture studies, bacteria are typically grown below 36 °C to prevent cell denaturation. However, higher rates of denitrification were observed for soil-slurry samples incubated at 45 °C than at 25 °C. Furthermore, optimum temperature ranges for denitrification as reported in the literature can be as high as 65 °C. Answers to the question of whether denitrification at this high temperature is a consequence of abiotic processes (chemodenitrification) or of thermophilic bacteria activities would help us to understand and better quantify the process.

The Arrhenius equation has traditionally been used to express the temperature dependance of processes such as mineralization and denitrification. As seen here, however, the Arrhenius equation does not take into account the nutritional status of the soil. Different Q_{10} values were obtained when the same soil was incubated using different carbon sources and concentrations. Alternatives or amendments to this equation may be necessary as well.

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BIOGRAPHICAL SKETCH

Leonel Alejandro Espinoza Guzman was born on February 1, 1960 in the capital city of Tegucigalpa, Honduras. In 1978 he entered the Universidad Nacional Autonoma de Honduras. However, his love for agriculture moved him to enroll the following year in Escuela Agricola Panamericana, "El Zamorano", where he received the diploma of "Agronomo" in 1981.

In 1984, Leonel was awarded a scholarship from the Organization of American States (OAS) to continue his studies in the United States. He enrolled at Iowa State University in the fall of 1984, where he received a Bachelor of Science degree in Agronomy in May 1986.

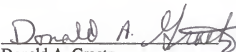
In August 1990, Leonel was awarded a research assistantship from the Horticultural Science Department of the University of Florida. He received a Master of Science degree in the fall of 1992. On January 1993, Leonel started his doctoral studies in the Soil and Water Science Department of the University of Florida, where he is presently enrolled.

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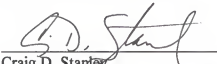
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August, 1997


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